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Modulation of the Mirror Neuron System during action observation: neuroimaging evidence for experience-dependent motor resonance in healthy humans and children with Unilateral Cerebral Palsy

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Declaration by Author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my PhD candidature.

I acknowledge that an electronic copy of my thesis must be lodged with the University of Parma D-Space and subject to the policy and procedures of the University of Parma.

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Dedicated To my Brother & my Sister

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Abstract

It is now a well-accepted notion in neurophysiology that the observation of actions performed by others activates in the perceiver a set of neural structures responsible for the actual execution of those same actions. Indeed, action observation is believed to activate the mirror neuron system (MNS), formed by visuomotor neurons which fire both when individuals perform a goal-directed action or when they observe a similar act, performed by another individual. The function of this network is to transform the sensory representations of observed motor acts into the corresponding motor representations. Overall, action observation is considered an effective tool to enhance motor learning in healthy people and patients with stroke or Parkinson disease. The action observation therapy has been also used in children with Unilateral Cerebral Palsy (UCP). An important assumption for an observation-based therapy/training is the plasticity of the MNS. Several studies indicate a strong role of the MNS in representing previously acquired motor skills, showing that, during action observation, observers tend to "resonate" more strongly with observed actions already embodied in their own motor repertoire. Here, we hypothesized that the MNS could be better activated: 1) in healthy humans by the observation of a model with a motor repertoire similar to that of the observer; 2) in UCP children by a paretic hand model as compared to a healthy one. The first fMRI study concerns the role of motor experience in the modulation of MNS in healthy humans observing complex manipulative actions performed with different levels of expertise. The hypothesis was that the MNS is more active when the observed hand actions correspond to the own motor expertise. Eighteen healthy volunteers, without particular hand motor skills, observed video-clips displaying hand-object manipulation performed with the right hand, in a first person perspective. The manipulation was performed by an expert with a high level of manual/fingers dexterity, by an actor with an intermediate ability or by a naïve subject. The most important result was that the observation of actions performed by a naïve model produced a stronger activation in a dorsal parieto-premotor network of areas, mostly lateralized to the left hemisphere. Secondly, the results showed that the superior parietal lobule was functionally coupled with bilateral parietal and frontal areas including the dorsal premotor cortex. This could suggest that this dorsal circuit, possibly involved in the processing of kinematic proprieties of the observed action, tends to resonate strongly with actions already embodied in the personal motor repertoire. In the second fMRI study, a group of UCP children, homogeneous for type of hand impairment and lesion side, observed grasping actions performed

by a healthy or a paretic hand and their brain activations were compared with that of a group of typically developing (TD) children matched by age. The results showed that the MNS areas were activated not only in TD, but also in UCP children, in spite of the presence of extended lesions. According with the hypothesis, the main result was that in UCP children the MNS was more activated during observation of actions performed by the paretic hand model with respect to the healthy one, suggesting that this neural system was modulated by the degree of similarity between observer’s motor repertoire and observed model. A possible explanation is that in UCP children the MNS is sensitive not only to the goal of the action but also to observed action kinematics. In situations in which the observed action does not belong to the personal motor repertoire, deviation in kinematics could be fundamental to explain a decreased motor resonance with the observed model. In a translational medicine perspective, these results could allow the therapy to achieve a greater motor improvement and to better adapt it to individual upper limb clinical characteristics.

Abstract (Italian)

Numerose evidenze neurofisiologiche confermano che l’osservazione delle azioni è in grado di attivare gli stessi circuiti neurali responsabili della loro esecuzione. Tale meccanismo si basa sull’attività dei neuroni specchio (MNS), una classe di neuroni visuo-motori che si attivano sia durante l’esecuzione un atto motorio finalizzato, sia durante l’osservazione di un altro individuo che esegue atti motori analoghi. La funzione di questo sistema è quella di trasformare le rappresentazioni sensoriali degli atti motori osservati nella loro rappresentazione motoria. Recentemente, l’osservazione-imitazione delle azioni (AOT) è stata utilizzata come strategia di apprendimento motorio in individui sani e pazienti con disturbi neurologici, quali Parkinson e Stroke. Inoltre, l’AOT è stata applicata con successo nella riabilitazione motoria di bambini affetti da paralisi cerebrali emiplegiche (UCP). Studi recenti hanno dimostrato che il MNS tende ad essere maggiormente attivato durante l’osservazione di azioni che fanno parte del repertorio motorio dell’osservatore, come le azioni della vita quotidiana. Sulla base di questo assunto, è possibile ipotizzare che il sistema specchio può essere maggiormente attivo 1) in individui sani che osservano azioni che fanno parte del proprio repertorio; 2) in bambini con UCP, durante l’osservazione di un modello patologico, rispetto a un modello sano. In questa tesi sono riportati i risultati di due esperimenti di MRI funzionale. Il primo studio era finalizzato a indagare il ruolo dell’esperienza motoria nella modulazione del MNS, in soggetti volontari sani, mediante l’osservazione di azioni di manipolazione eseguite con differenti livelli di expertise. I partecipanti erano 18 volontari sani, senza particolari abilità di manipolazione o altri expertise motori. Il compito era quello di osservare passivamente dei video che riproducevano la manipolazione di oggetti in prima-persona, eseguita da un attore professionista con un livello elevato di destrezza manuale, da un attore con capacità manuale intermedia, o da un attore naïve. I risultati hanno evidenziato che l’osservazione delle azioni eseguite dal modello naïve producevano un pattern di attivazione più esteso nelle aree del MNS, rispetto all’osservazione degli altri modelli. Inoltre, dai risultati è emerso che il lobulo parietale superiore mostrava una connettività funzionale con altre aree parieto-frontali dorsali, tra cui la corteccia premotoria dorsale. Questo risultato suggerisce che tali aree implicate nel processing delle proprietà cinematiche potrebbe svolgere un ruolo chiave nell’elaborazione delle azioni che fanno parte del proprio repertorio motorio. Nel secondo studio, un gruppo di bambini con UCP, omogeneo per livello di impairment motorio e per localizzazione delle lesioni cerebrali, è stato sottoposto a un

esperimento fMRI in cui era richiesto di osservare passivamente delle azioni di raggiungimento e afferramento di oggetti, eseguite da un altro bambino emiplegico (con un livello di impairment motorio simile a quello dei partecipanti), oppure da un bambino sano. Inoltre, le attivazioni del bambini UCP sono state confrontate con quelle di un gruppo di bambini con sviluppo tipico. Dai risultati di questo studio è emerso che i il MNS era attivato non solo nei bambini sani, ma anche nei pazienti UCP, nonostante la riorganizzazione del sistema sensorimotorio. In accordo con l'ipotesi, il risultato principale dimostra che il MNS è in grado di coordinare l'informazione visiva con l'esperienza motoria dell'osservatore. Da una prospettiva di medicina traslazionale, questi risultati permettono di personalizzare la terapia basata sull'osservazione, al fine di raggiungere un miglior controllo motorio dell'arto superiore.

1. GENERAL INTRODUCTION

1.1 The mirror neuron mechanism

1.1.1 Functional organization of the cortical motor system in primates

In the last three decades our view of the cortical motor system has radically changed. Traditionally three main motor areas were described, namely the primary motor area (M1) devoted to control movement execution, the premotor region and the supplementary motor area (SMA) involved in motor preparation. More recently, new functional and neuroanatomical data have been collected that give a different picture of the cortical organization of the motor system on the basis of cytoarchitectural and functional data. The premotor cortex has been shown to be subdivided in several sub-areas that, according to Matelli et al. (1985; 1991) are labeled with the letter F followed by Arabic numbers (F2-F7). Each subarea of this mosaic is characterized by a specific pattern of connections with other parietal and prefrontal areas and also with subcortical structures, in particular the spinal cord. A modern map of the monkey motor cortex based on the works of Matelli and coworkers is shown in the Figure 1. The areas F1-F5 are mainly connected with parietal areas, while F6-F7 are mainly connected with the prefrontal cortex. Parietal-related areas send projections to the spinal cord but this is not true for prefrontal-related areas. In addition, several distinct body parts are represented in each subdivision of the premotor cortex. Summing up, the core of the cortical motor system is now considered as a series of parallel parieto-frontal circuits connecting specific areas of the frontal and parietal cortex which are devoted to specific sensorimotor transformations necessary for acting in the environment. These parieto-frontal circuits are influenced by attentional and motivational factors, possibly fed up by the dorsal prefrontal cortex (Gerbella et al. 2017; Rozzi and Fogassi, 2017).

To execute an action (e.g., grasping a bottle of wine and pour it into a glass), one must have a prior intention (to pour out the wine), select a sequence of motor acts (reaching, grasping the bottle, turn it to the glass and pour out the wine) and finally execute this sequence. This view on action organization implies different hierarchical levels: movements, motor acts, actions. For achieving the action final goal (motor intention), single elements must be linked one to the other according to a precise temporal structure.

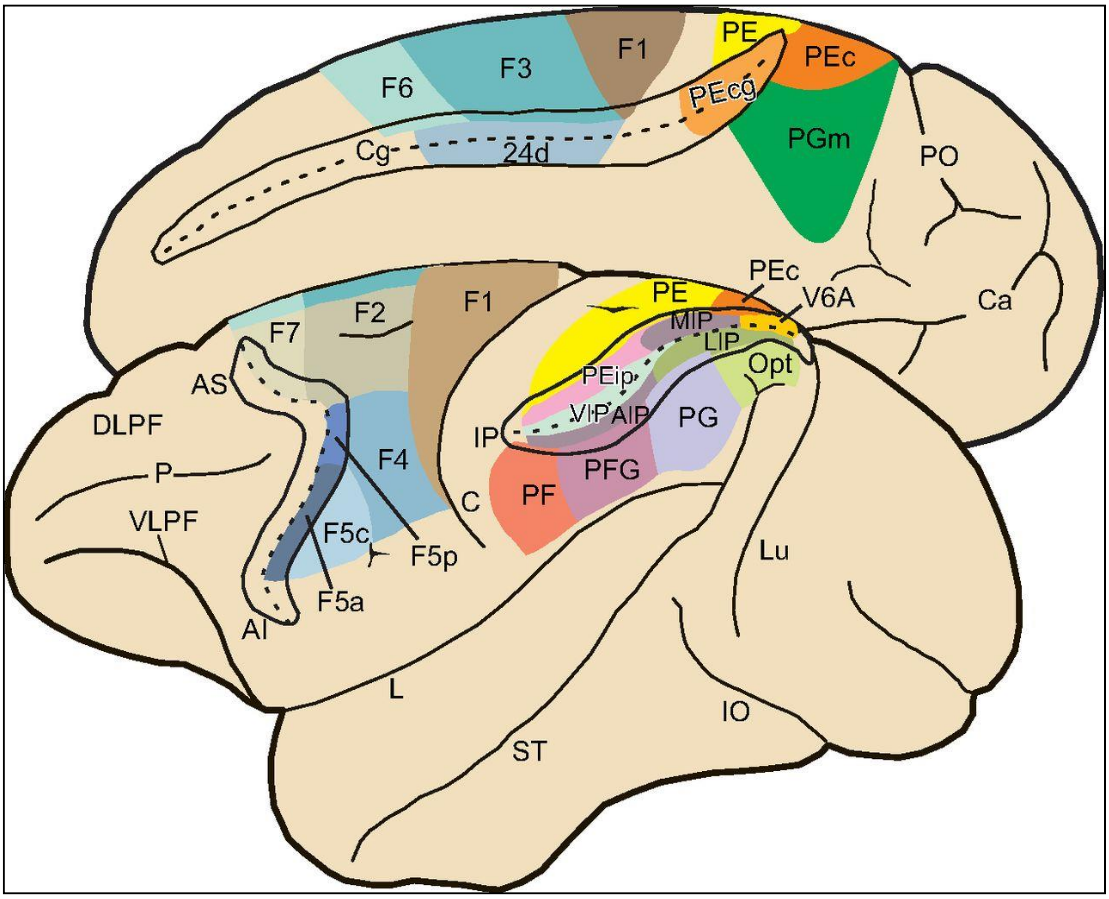


Figure 1. Lateral and mesial view of the monkey brain showing the subdivisions of the agranular frontal and posterior parietal cortices. The intraparietal and arcuate sulci have been opened to show the areas buried inside them. AI, inferior arcuate sulcus; AIP, anterior intraparietal area; AS, superior arcuate sulcus; C, central sulcus; DLPF, dorsolateral prefrontal cortex; FEF, frontal eye field; IO, inferior occipital sulcus; L, lateral fissure; LIP, lateral intraparietal area; Lu, lunate sulcus; MIP, medial intraparietal area; P, principal sulcus; ST, superior temporal sulcus; VIP, ventral intraparietal area; VLPF, ventrolateral prefrontal cortex. Adapted with permission from Rizzolatti et al. (2014).

While area F1 is crucial for movement execution, area F5 plays a fundamental role in encoding hand and mouth motor acts (Ferrari et al. 2003; Gentilucci et al. 1988; Kurata and Tanji 1986; Rizzolatti et al. 1988). This statement is based on single neuron studies that showed that most F5 purely motor neurons encode specific motor acts such as grasping, breaking, holding, rather than the individual movements that form them (Rizzolatti et al. 1988). Neurons discharging for a specific motor act typically do not discharge during the execution of similar movements aimed at a different goal, for example, a neuron that discharges during hand movements for grasping a cup of coffee does not discharge during similar movements aimed at pulling it. Furthermore, some F5 neurons discharge when the same goal is achieved by using different effectors (e.g., the right hand, the left hand or the mouth), thus requiring a completely different set of movements. Many neurons code specific types of grip, such as precision grip, whole hand prehension, or finger prehension. Finally, concerning the timing of grasping, some neurons discharge during the whole motor act (e.g., opening and closure of the hand), while others only during a certain phase of it (e.g., shaping of the hand for taking possession of the object).

On these bases, it has been proposed that F5 contains a “vocabulary” of motor acts (Rizzolatti et al. 1988). This motor vocabulary is constituted of “words,” each of which is represented by a population of F5 neurons. Some of them encode the general goal of a motor act, others encode how a specific goal-directed motor act must be executed, and others specify the temporal aspects of the motor act to be executed (Jeanneroad et al. 1995). A further demonstration that F5 neurons encode motor acts has been recently provided by a study in which the same motor goal (i.e., taking possession of food) was achieved by means of opposite movements (Umiltà et al. 2008). In the study by Umiltà and coworkers, monkeys grasped objects using normal pliers, which require hand closure to take possession of the object, and reverse pliers, that instead require hand opening to achieve the same goal. Authors demonstrated that most of the F5 neurons encode goal achievement (e.g., taking possession of the target) independent of the specific fingers movement (flexion or extension) required to achieve it.

Another recent study (Belmalih et al. 2009) demonstrated that area F5 comprehends three architectonically distinct sectors, probably corresponding to functionally different areas (see Figure 1). Two of these sectors are located within the inferior postarcuate bank, one more posteriorly (F5p) and the other more anteriorly (F5a). The third sector occupies most of the postarcuate convexity (F5c). Both F5p and F5c are densely anatomically connected with the parietal cortex and in particular with the areas AIP, PF, PFG, and SII (Petrides, 1984; Matelli et al. 1986; Luppino et al. 1999; Borra et al. 2008; Gerbella et al. 2010).

In addition to purely motor neurons, area F5 contains neurons also responding to sensory stimuli. Many of them respond to somatosensory stimuli, but a consistent percentage (25%) is responsive to the presentation of visual stimuli (Rizzolatti et al. 1988). Among these latter, a set discharges in response to the presentation of 3D objects (Murata et al. 1997). These neurons are called canonical neurons. They are mostly located in area F5p. Canonical neurons are activated by objects of a specific size, shape, and orientation. Visual and motor object specificities are congruent, that is a neuron motorically selective for a specific grip shows a specific visual response during presentation of an object whose shape is congruent with that grip. There is evidence that the response of canonical neurons to the presentation of visual stimuli cannot be accounted for in terms of motor preparation. In fact, these neurons also respond when the monkey is not required to grasp the presented object, but simply to observe it (Murata et al. 1997). The interpretation of the discharge of canonical neurons is that these neurons encode the visual stimulus in a motoric manner: when an object is seen, the discharge of canonical neurons encode a potential motor act, congruent with the properties of the presented object, independently of whether the act will be executed or not.

F5p is strongly connected with the anterior intraparietal area (AIP) (Luppino et al. 1999; Borra et al. 2008; Gerbella et al. 2010). The functional properties of this area have been extensively studied by Sakata and co-workers (Murata et al. 2000; Sakata et al. 1995; Taira et al. 1990). AIP neurons can be subdivided into three main classes: “motor-dominant,” “visual and motor,” and “visual-dominant” neurons. In particular, motor-dominant neurons discharge during grasping and holding both when the grasping action is performed in light and when it is executed in complete darkness; on the contrary, they do not discharge during object fixation. Visual-dominant neurons become active when grasping is performed in light, but not in dark. They respond to simple object fixation. Finally, visual and motor neurons discharge during object fixation and during grasping in light and in dark (Murata et al. 2000).

One could raise the question: how do visuomotor transformations for grasping occur in this circuit? There are various models that try to explain the role that AIP and F5 play in this process (Taira et al. 1990; Jannerod et al. 1995). A common idea underlying these models is that when an object is observed, AIP neurons extract specific aspects of its physical properties (affordances concept by Gibson 1979) and provide F5 with the description of the possible ways in which the object could be grasped. On the basis of the intention of the individual and the context, the prefrontal lobe selects AIP visuomotor neurons and neurons in F5 that code the most appropriate grip. The information relative to the chosen grip is then sent from F5 to F1, where the different movements necessary to grasp the object are selected and the final command for execution is generated.

1.1.2 The mirror neuron system in monkey

Mirror neurons were described for the first time 20 years ago in the monkey ventral premotor area F5 (di Pellegrino et al., 1992; Rizzolatti et al., 1996; Gallese et al., 1996) and subsequently in the monkey inferior parietal area PFG (Fogassi et al., 2005; Rozzi et al., 2008). They are formed by a distinct class of motor neurons that motorically encode a specific motor behavior and, in addition, are activated by the observation of the same or a similar motor behavior. Thus an observed action produces, in the observer's brain, a motor activation, as if the observer was actually executing it. Single-cell recording studies have classically defined the action observation system (MNS) as comprehending three areas (Rizzolatti et al. 2014): the superior temporal sulcus (STS) in the temporal cortex (Perrett et al. 1989, 1990; Jellema and Perrett 2006), area F5 in the premotor cortex (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996; Umiltà, et al. 2001) and area PF/PFG in the inferior parietal cortex (Fogassi et al. 2005; Rozzi et al. 2008).

It is now well accepted that mirror neurons code the goal of motor acts (Rizzolatti et al. 2001). To test this hypothesis, two series of experiments were carried out. In the first, mirror neurons that responded exclusively during the observation of the late phase of grasping and / or during object holding were tested in two conditions. In one, the monkey saw the hand of the experimenter grasping and holding an object (full vision condition). In the other, the monkey saw only the experimenter's hand moving toward a screen but the final critical part of the motor act (hidden condition). The results showed that more than half of the F5 mirror neurons discharged in the hidden condition. This demonstrated that is the meaning of the observed actions, and not the vision of it, that trigger mirror neurons response (Umiltà et al. 2001).

A second study was performed using different modality, for example, auditory stimuli typical of certain motor acts presented to the monkey (Kohler et al. 2002). The results of this work showed that a large number of the recorded mirror neurons responded not only to the observation of specific motor acts (e.g. breaking a peanut), but also to their sound. By contrast, other types of auditory stimuli related to different motor acts or unspecific sound-like white noise, were not effective. Taken together, These findings clearly supports the notion that mirror neuron responses code the goal of motor acts performed by others, also in the absence of visual cues. These cues trigger mirror neurons only insomuch as they allow the understanding the goal of motor act. When goal comprehension is possible on other bases, mirror neurons are able to signal it, even in the absence of visual stimuli.

Another cortical region traditionally considered part of the mirror neuron circuit is the superior temporal sulcus (STS). It represents the major input to the IPL, in turn connected to ventral premotor cortex. In the rostral part of the ventral bank of STS, some neurons were described that fired during observation of hand grasping (Perrett et al. 1989). However, neurons in STS do not discharge during active movements. It seems reasonable to argue that STS is the origin of the higher-order visual input necessary for building mirror neurons in the parieto-frontal circuit. This point has been recently investigated using fMRI complemented by neuroanatomical tracing techniques in the monkey (Nelissen et al. 2011).

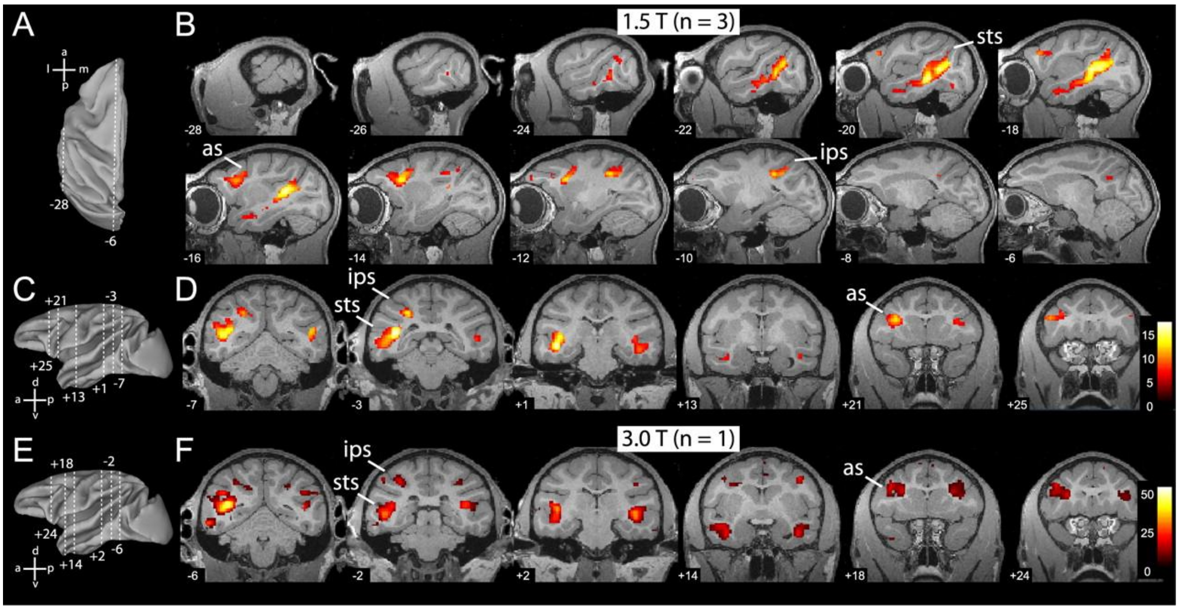


Figure 2: Overview of MRI brain activations (recorded with 1.5/3T fMRI) during the observation of grasping acts (Nelissen et al. 2011). as, arcuate sulcus; ips, intraparietal sulcus; sts, superior temporal sulcus.

In their fMRI experiments, video-clips of grasping actions performed by a human actor were presented. The results showed activation in three nodes (see Figure 2): STS, IPL and the arcuate region. A subsequent region of interest (ROI) analysis, based on the contrast between grasping action and a variety of static stimuli, showed that in the parietal lobe action-related increase in discharge was present in areas PFG and AIP. No action-specific activation was found in other parietal areas. Action-related stimuli evoked stronger activation than static stimuli in all STS areas.

The same motor act may be part of different actions having different final behavioral goals. For example, a glass of wine grasped by its body indicates the desire to drink the wine, while the same glass grasped by the top suggests the intention to move the glass. Even when two grips are identical, one may guess the intention of the agent from the object the agent is grasping or from the context. According to this view, it has been proposed that observation of a motor act done by another individual allows the observer not only to understand the goal of the observed motor act, but often also the intention behind it. Recently, the issue of how the intention of an action, i.e., the overarching goal of a series of motor acts, is encoded in the nervous system has been studied in PFG and in F5, (Fogassi et al. 2005; Bonini et al. 2010). The first experiments trying to assess a possible relation between motor intention and mirror activity were carried out in the inferior parietal lobule, mostly in area PFG. Neurons discharging during the execution of grasping motor acts were tested in two main conditions (Fogassi et al. 2005). In the first condition, the monkey had to reach and grasp a piece of food and bring it to the mouth; in the second, the monkey had to reach and grasp an object and place it into a container. Moreover, in the first condition, the monkey was allowed to eat the grasped food while, in the second one, it was rewarded with a piece of food given to it by the experimenter. The results showed that the majority of the recorded neurons discharged in accordance with the action in which grasping was embedded. Of them, the great majority (73%) preferred grasping for eating. In order to control that the differential discharge of neurons during the same motor act performed in the two conditions was not due to a mere difference in the stimuli to be grasped, monkeys were trained to grasp the same piece of food in both conditions. The results showed that neuron selectivity remained the same.

Several studies have shown that programming of a motor act should consider the constraints posed by the target of the subsequent motor act. In the experiment described above, the kinematics of the reach-to-grasp motor act was measured. It was found that this motor act, when followed by arm flexion was faster than when the same motor act was followed by arm abduction. Furthermore, in a subsequent study, neurons were recorded from both F5 and the IPL (area PFG) in order to compare the activity of F5 and PFG in motor sequence organization (Bonini et al. 2010). The same conditions as in the study described above were used. The results confirmed that a large number of F5 neurons code grasping according to the goal of the action in which it is embedded. The same effect was found also in PFG. It may sound surprising that most IPL neurons discharge depending on the final action goal. An important aspect of motor organization to consider is the fluidity of motor acts. This fluidity is necessary, because action execution should occur without any gap. The results of the above-described studies suggest that neurons encoding specific motor acts within an action form pre-wired intentional chains, in which a neuron encoding a motor act is facilitated by the neuron encoding the previous one.

Thus, when an action constrained neuron became active it triggers the whole motor chain in the observer that may grasp the agent intention.

1.1.3 The human mirror neuron system

A large number of studies based on non-invasive electrophysiological (e.g. EEG, magnetoencephalography (MEG), transcranial magnetic stimulation (TMS) or brain imaging (e.g. PET, functional MRI (fMRI) techniques demonstrated the existence of the mirror mechanism in humans (Fadiga et al., 1995; Rizzolatti et al. 1996b; Grafton et al. 1996; Grezes et al. 1999; Iacoboni et al. 1999; Buccino et al. 2001; Iacoboni et al. 2001; Koski et al. 2002; Buccino et al. 2004a; Buccino et al. 2004b; Gazzola et al. 2007a; Gazzola and Keysers 2009; Gatti et al. 2017). Brain imaging studies (see Caspers et al. 2010; Molemberghs et al. 2012) have enabled to locate the mirror areas. These studies showed that the observation of transitive actions done by others results in an increase in blood oxygen level-dependent (BOLD) signal not only in visual areas, but also in the IPL and the ventral premotor cortex, as well as the caudal part of the inferior frontal gyrus. These latter three areas have motor properties and closely correspond to the areas that contain mirror neurons in the monkey (Figure 3).

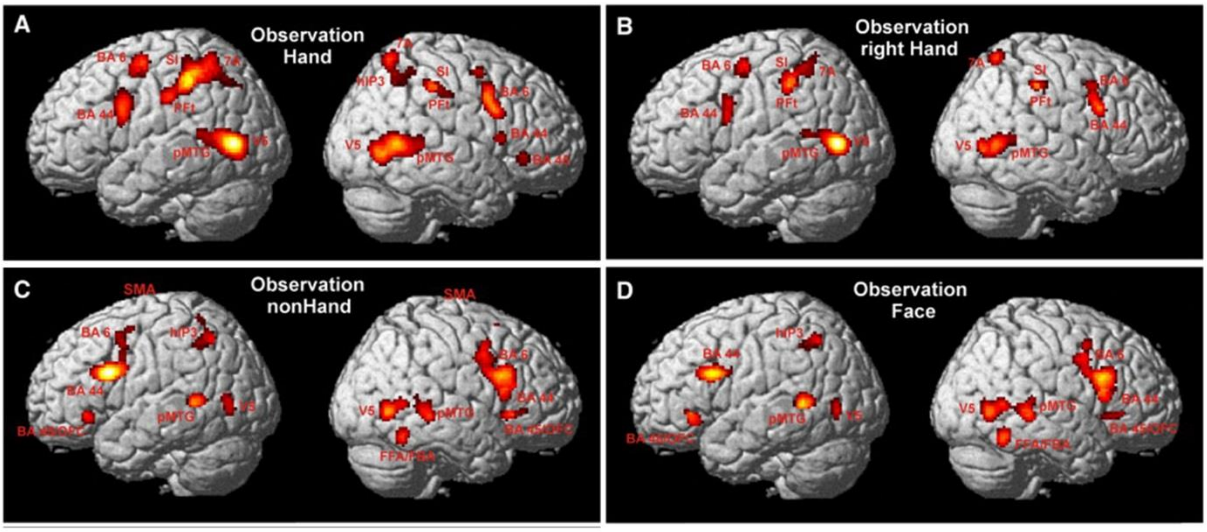


Figure 3: Meta-analysis (Caspers et al. 2010) showing the activations during action observation. Results from meta-analysis are displayed on the left and right lateral surface view of the MNI single subject EPI template.

The observation of single body part movements (transitive and intransitive actions) activated the premotor cortex in a somatotopic manner (Buccino et al. 2001). In an fMRI study, Buccino and coworkers (2001) presented normal volunteers with video clips showing transitive motor acts performed with different effectors, as mouth, hand, and foot. The activations during the observation of these motor acts were contrasted with those obtained during the observation of the same stimuli presented statically. They found a trend towards a somatotopic organization both in the parietal and in the frontal lobes, although with considerable overlap between the body-parts representations. A subsequent study (Wheathon et al. 2004) used meaningless intransitive movements of the face, hand, or leg and found a similar somatotopic arrangement in the frontal and parietal lobes, but present only in the right hemisphere. More recently, Abdollahi and coworkers (2012) investigated the cortical representation of manipulation, locomotion, and climbing. The result showed that observation of climbing activated the rostro-dorsal part of superior parietal lobule, locomotion the same region, although weakly, and finally, manipulation the area homologue of monkey AIP. Moreover, a study addressed this issue categorizing the observed movements in distal limb, proximal limb, and axial movements (Sakreida et al. 2005). It was found that axial movements activated the medial premotor cortex (SMA), proximal limb movements activated the dorsal part of the convexity of the premotor cortex and, finally, distal limb movements activated ventral premotor cortex.

Other neuroimaging studies investigated the overlap of areas that are activated both during action observation and execution. Gazzola and Keysers (2009), using single-subject analyses of unsmoothed fMRI data showed that voxels shared between action observation and action execution were located, in addition to the classical parieto-premotor circuit, also in various other cortical areas, namely, dorsal premotor, middle cingulate, somatosensory, superior parietal, and middle temporal cortex (see Figure 4). Authors argued that it is plausible that the activations outside the classical MNS reflect sensory predictions from internal models. These activations would enrich the information about other individuals’ actions that the mirror mechanism provides.

In another fMRI study, Biagi et al. (2010) investigated the activation of human area AIP during the observation of complex object-manipulation tasks (e.g. inserting a key in a lock and turning it) as compared to simple tasks (whole hand grasping of an object) executed with the left and the right hand in a first person perspective. Their results showed that, in general, both complex and simple tasks produced an activation of the fronto-parietal mirror system and that the activity of AIP in each hemisphere was higher during observation of the contralateral hand (hand identity effect). A Region of Interest (ROI) analysis of the parietal activations related to hand identity showed that each AIP was more active during the observation of complex with respect to simple tasks. In the right AIP this effect was stronger during observation of the contralateral hand, in the left AIP was equally strong during observation of both hands. This complexity-related property was not observed in the other activated areas. These findings support the concept that the observation of motor acts retrieves the internal representation of those same acts in the observer's motor system (direct-matching hypothesis based on the mirror neuron mechanism).

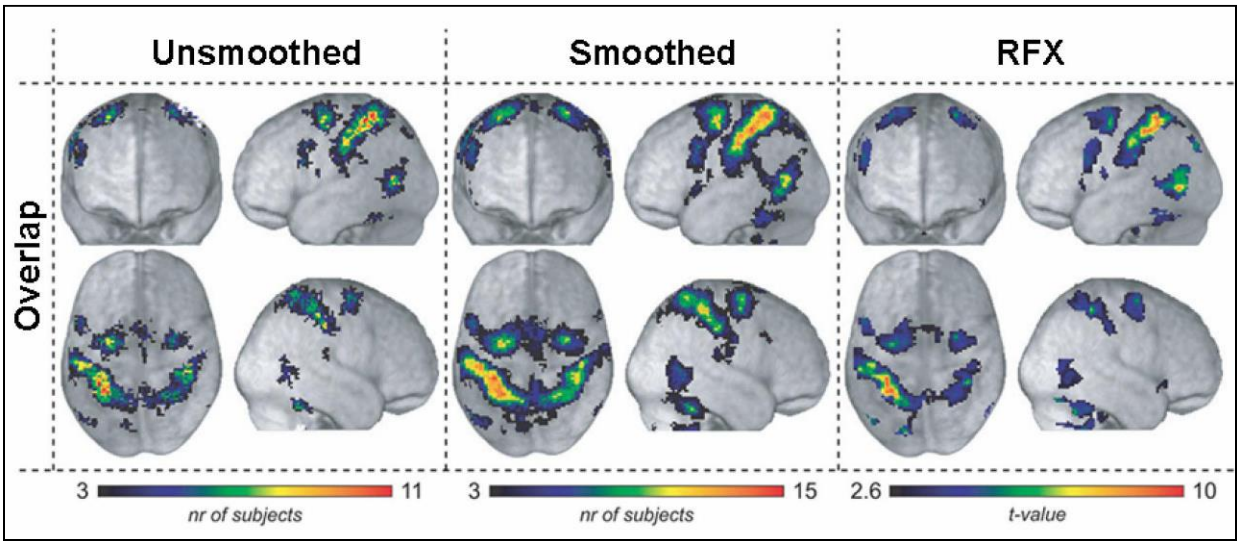


Figure 4. Human brain regions with mirror properties. Images within the left and the middle columns show the number of subjects showing 'mirror' regions using unsmoothed and smoothed data respectively. Images within the right column show the t-values of a traditional random effect analysis (RFX) using smoothed data. (Modified from Gazzola & Keysers, 2009).

Concerning the classical distinction in motor organization between the two parallel "modules" of reaching and grasping (see Jeannerod 1997), several human neuroimaging studies have investigated observation of grasping or of object manipulation (Grafton et al. 1996; Binkofski et al. 1999; Buccino et al. 2001; Grèzes et al. 2003; Johnson-Frey et al. 2003; Tai et al. 2004). Other studies have compared grasping observation with grasping execution or imitation (Rizzolatti et al. 1996b; Hamzei et al. 2003; see also Nishitani and Hari 2000), observation versus imitation of simple finger movements (Iacoboni et al. 1999), or observation versus execution of more complex finger movements, such as playing guitar strings (Buccino et al. 2004b). Typically studies that involve less of the arm transport phase in both observation and execution conditions (e.g. Rizzolatti et al. 1996, where only the final phase of the hand grasping an object was viewed) find activations in the inferior frontal gyrus and the IPL, but not the SPL. Tasks that involve a greater arm transport phase (e.g. Hamzei et al. 2003, where a cup was grasped and moved from the lap to the mouth; Grafton et al. 1996; Culham et al. 2003) do activate the SPL. Whereas previous studies have mostly examined hand actions related to grasping, hand–object interactions, or local finger movements, only few studies investigated pure reaching (i.e. the transport phase of the hand to a particular location in space), without grasping (Filimon et al. 2007; Di Dio et al. 2013; Malfait et al. 2010). These studies found activations in a dorsal parieto-frontal circuit spatially overlapping with the areas activated during reaching execution. For example, Filimon and coworkers (2007) used fMRI to map the cortical representations of executed reaching, observed reaching, and imagined reaching in humans. They reported activations for observed reaching located more dorsal than activations typically reported in the literature for observation of hand–object interactions (grasping) in human and monkey. The results suggest that the human MNS is specific to the type of motor act performed, and that these parieto-frontal activations for reaching are a putative human homologue of the neural circuits underlying reaching motor acts in the monkey.

1.1.4 Mirror activity is modulated by motor experience

Experience of a certain motor skill appears to be of importance in modulating the MNS responses to observation of actions requiring that skill. In an fMRI study (Buccino et al. 2004a), it has been investigated whether the human putative MNS is activated by the observation of actions performed by different species. Participants were presented with mouth actions related either to food ingestion (biting) or to communication. These actions were performed by a human being, a monkey and a dog. The results showed that the observation of biting activates the premotor cortex and the inferior parietal lobule, regardless of the observed species, whereas the observation of communicative actions was effective in recruiting the premotor cortex and the inferior frontal gyrus (Broca’s region) only when participants observed a conspecific (human being moving the lips as during speaking), but not when they observed a communicative gesture performed by a monkey or a dog. These findings have been interpreted as proof that the human putative MNS can match an observedaction on the neural structures involved in its execution only if the observed action belongs to the observer’s motor repertoire (actions that observer can perform). Similarly, the motor expertise of the observer affects the recruitment of human putative MNS.

Other evidence came from the studies of specific populations of experts, (e.g., dancers, elite sportsmen) who are skilled in determinate actions. Experiments dealing with mirroring in hyperspecialized population tried to disentangle the contribution of motor and visual expertise. For example, Calvo-Merino and coworkers (2005) investigated the mirror responses of three different groups of participants: classical dancers, teachers of Capoeira, and people naive to dancing. Stimuli consisted in videos of either Capoeira or classical dance steps (Figure 5).

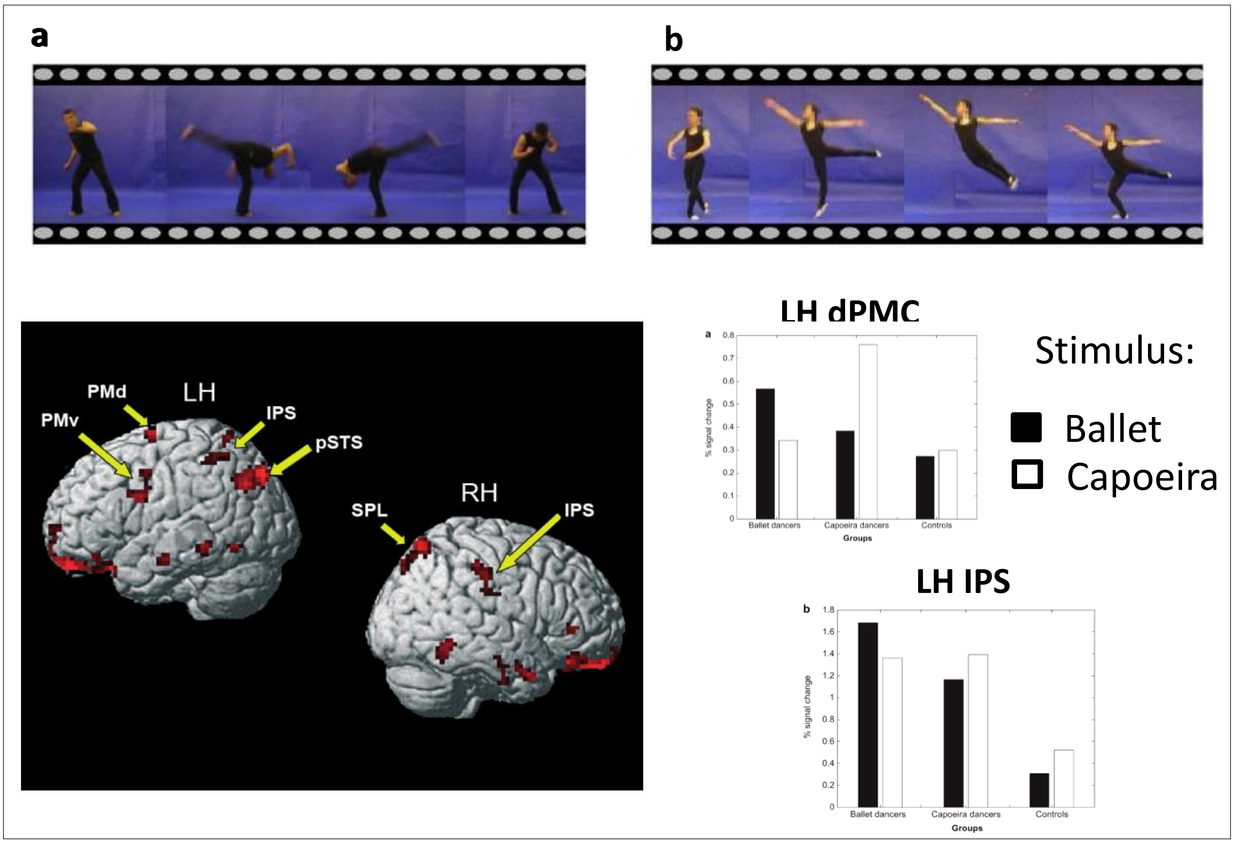


Figure 5: Stimuli in the study of Calvo-Merino and collegues (2005). Color videos of standard classical ballet and capoeira movements were performed by professional dancers. Twelve different moves of each style (a, capoeira; b, ballet) were matched by a professional choreographer for kinematic features. The rendering template represents the effects of motor expertise on brain responses to action observation defined as the group by condition interaction. Bars graphs show parameter estimates for the influence of motor expertise on action observation in the central voxels of areas classically identified with the human mirror system: dorsal premotor cortex (dPMC, -24 -6 72), left intraparietal sulcus (IPS, -33 -45 54).

A clear double dissociation was found between the two expert groups. Mirror responses to Capoeira steps were stronger in the Capoeira experts, and vice versa, mirror responses to classical dance steps were stronger in ballet experts. In a further experiments, the same researchers (2006) tried to disentangle the visual familiarity with the dance steps from the motor expertise. They studied classical ballet and examined the activation in men and women determined by the observation of steps done by dancers of the same and different gender. They found that the MNS was activated more strongly by steps executed by individuals of the same gender of the observer. This finding indicates that the motor expertise and not visual experience is crucial in activating the mirror system.

The findings by Calvo-Merino were extended in a subsequent study by Cross and coworkers (2009). In their study, naïve participants were trained for 5 days on dance sequences set to music videos. Each day, participants physically rehearsed one set of dance sequences, and passively watched a different set of sequences. Functional magnetic resonance imaging was obtained prior to and immediately following the 5 days of training. fMRI was weekly recorded while dancers observed the new dance sequences. The results showed that the observation of another dancer’s movements activated the MNS circuit. Critically, the activation of IPL and premotor cortex was sustained during observation of sequences that were danced, but declined for unfamiliar sequences, relative to the pre-training scan session. These results demonstrated the emergence of action resonance processes in the human brain based on observational learning without physical practice and identify commonalities in the neural substrates for physical and observational learning. The modulation by own personal motor experience on MNS activity was also investigated by Van Elk and coworkers (2008), extending previous findings based on expert motor skills in adults to the natural development of actions in infants. They presented to 14- to 16-mo-old infants videos of other infants who were crawling or walking. The results showed a stronger desynchronization in both the mu- and the beta frequency bands for observation of crawling compared to walking. Second, the amount of crawling experience was directly related to the degree of mu- and beta-desynchronizations. The strong correlation between crawling experience and mu and beta-desynchronizations provided support for the view that motor resonance during action observation is directly related to infants' natural developing action abilities. Overall, these findings clearly indicate that the motor experience has a significant influence on the MNS responses.

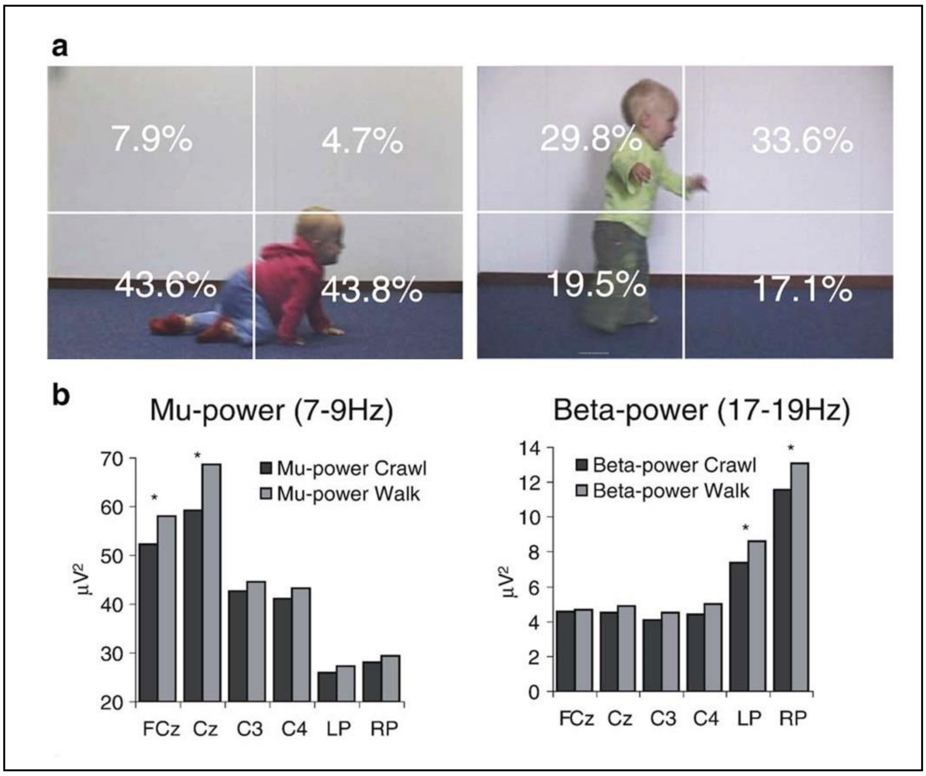


Figure 6: Different EEG reactivity in response to crawling and walking in infants. **a)** relative percentage of looking time for designated areas of interest for crawling (*left*) and walking videos (*right*). Values indicate looking times for observation the videos. **b)** grand averaged EEG power for walking videos (dark bars) and crawling videos (light bars) at different electrode sites (abscissae) for the mu-frequency band (7–9 Hz) and the beta-frequency band (17–19 Hz). Ordinate: voltage.

1.1.5 From goals to kinematics: the hierarchy of motor control

As just mentioned before, the MNS match the observed actions with the motor representations belonging the personal motor vocabulary. However it is not clear if the MNS resonates more strongly with the kinematic features of the observed movements or only with the final goal of the action. Evidence for goal processing in human motor areas comes from a series of fMRI studies. First evidence in this sense derives from the study of movements performed by artificial effectors. Gazzola and coworkers (2007a) presented participants with video-clips of a human or a robot arm grasping objects. The data showed that in spite of shape and kinematics differences, the parieto-frontal MNS became active in both conditions. This study was extended by Peeters and coworkers (2009) who studies in human and monkeys the cortical activations during the observation of motor acts executed by a human agent, a robot agent or performed with a tool. Their findings showed that in both species, the observation of an action, regardless of how performed, activated occipito-temporal, intraparietal, and ventral premotor cortex, bilaterally. In humans, the observation of actions done with simple tools yielded an additional, specific activation of a rostral sector of the left IPL. This latter site was considered human-specific, as it was not observed in monkey IPL for any of the tool videos presented, even after monkeys had become proficient in using a rake or pliers through extensive training.

In another TMS study, Senna and coworkers (2013) recorded MEPs from hand (FDI) and foot (AH) muscles, induced by sTMS delivered to the hand or foot motor areas, respectively, during the observation of actions performed by different effectors (i.e., hand or foot). They found that the excitability of the hand motor area was modulated not only by actions performed by the hand but also by grasping actions in general, regardless of the effector used to execute them. Likewise, the foot motor area was modulated not only by actions performed by the foot but also by the observation of either the foot or the hand pressing a foot pedal. The observation of an action unspecific for both hand and foot (i.e., stepping over) did not modulate specifically neither the hand nor the foot area. This evidence demonstrates that motor facilitation contingent upon action observation is not restricted to the same muscles involved in the observed action, but it occurs even in the muscles typically used to achieve the same action goal. Indeed, when a recognizable action goal (e.g., grasping) is performed through an unusual effector (e.g., the foot), a motor facilitation can be observed also in the muscles that are typically used to accomplish the observed action goal (i.e., the hand).

From a clinical point of view, a further evidence in favor of the goals representation in human MNS is provided by aplasic subjects. In the study of Gazzola and coworkers (2007b), two individuals born without arms and hands and normal controls were scanned while they were watching videos showing hand actions. In addition, the two aplasic individuals also made actions using their feet and mouth. It was found that in the aplasic individuals the parieto-frontal MNS active during the execution of foot and mouth movements was also active when they observed hand motor acts that they had never executed but the goals of which they could obtain using their feet or mouth. These findings were also confirmed by Aziz-Zadeh and coworkers, which found similar results in individuals with limb amputation (Liew et al. 2013).

On the other hand, a series of fMRI study used repetition suppression (RS) technique to distinguish the representation of goal from those related to the kinematic components of actions used to generate those outcomes. RS, also known as ‘fMRI-adaptation’, is based on a trial by trial reduction of a physiologic response to repeated stimuli. Repetition of a stimulus often results in a reduction of signal within the brain areas that encode that stimulus. Suppression occurs when two successive stimuli are represented within the same neural population, and release from suppression occurs when two successive stimuli are represented in different populations. Thus, the method assumes the existence of population coding within brain regions, for which there is extensive evidence in many parts of the cortex. In summary, RS effects allow to demonstrate regions selective for certain properties of the stimuli or to identify changes within a class of stimuli rather than between classes of stimuli.

Using this techniques, Hamilton and Grafton (2006, 2008) proposed a model to identify possible topologies in the human brain corresponding to a motor hierarchy. They tested this model using a library of stimulus sets, based on different observed features:

1. Kinematics. (a) Reach trajectory. RS at this level is related to the detection of how an agent is approaching an object. (b) Grip configuration. This level is defined by the specific hand-object interaction, such as a power or precision grip. (c) Means. This encompasses several features, including dynamic interactions based on object weight and the specific transport or manipulation of an object.

2. Goal-object. This is defined by the identity and function of the grasped object.

3. Outcome. This is defined by the physical consequences of an action, for example altering the position or configuration of objects in the world.

The results of these studies (Hamilton and Grafton 2006, 2008) point to the direction of a distributed network of areas within the MSN devoted to processing different features of the observed actions. In particular, selective processing within the MNS has been identified for basic perceptual features indicating *how* an observed action has been performed (e.g., kinematics, type of grip, trajectory) and for its goal (i.e., *what* has been observed). They argued that in terms of *how* an observed grasping action is performed, the coding of its basic kinematic features might occur within the lateral occipital and the superior precentral sulci, whereas, the trajectory assumed by the observed hand during reaching appears to be coded within the inferior and the middle occipital cortices, together with the middle IPS, the supplementary motor area, the middle frontal gyrus and the IFG. In terms of the goal subtending the observed action the suggestion is that the locus of such processing varies depending on the level of goal complexity. Sensitivity to the goal of a simple action (e.g., grasping a cup) has been identified mainly within the left IPS, but also to a lesser extent within the right IPS and left IFG (Hamilton and Grafton 2006; Grafton and Hamilton 2007). Sensitivity to the goal of more complex actions, which implies the modification of the state of an object in order to obtain a change in the environment (i.e., an outcome), has been associated with activity within the bilateral IPS and the IFG, particularly within the right hemisphere (Hamilton and Grafton 2008).

Moreover, in an fMRI study, Casile and coworkers (2010) demonstrate that regions in the left dorso-frontal and dorsal premotor cortex are differentially activated by observed human movements dependent on their compliance with the kinematic invariants of human movements (two-thirds power law). In their study, motor resonance with observed movements seems to be critically modulated not only by the appearance of the agent and the action goal but also by the consistency with the human kinematic laws of motion. This supports the notion that the MNS is important for analyzing the goal of the observed action on the basis of its biologically possible kinematics.

1.1.6 Imitation and motor learning

The basic circuit for imitation matches that for action observation, since it transforms the visual representation of observed actions processed by the STS region into a motor representation related to action goal and possibly into a kinesthetic representation at the level of the MNS (Iacoboni et al. 1999) whose output then become a motor commands for actual imitation. Contemporarily, an efference copy of the motor command is fed to the parietal cortex as a forward model of predicted sensory feedback that is compared, within the circuit, to the actual sensory feedback for online for motor control. The involvement of the MNS in imitation has been demonstrated by brain imaging studies. When subjects lift a finger in response to visual presentation of a target movement on a screen (imitation) or of a symbolic or spatial cue, activation is stronger during imitation than during the other motor conditions in the pars opercularis of the left IFG, the right anterior IPS, the right parietal operculum, and the right STS region (Iacoboni et al. 1999, 2001; Koski et al. 2002). Iacoboni and coworkers (1999) carried out an fMRI study in which there were two experimental conditions: *observation only* and *observation-execution*. In the first condition, participants were shown a moving finger, a cross on a stationary finger, or a cross on empty background. They were instructed to passively observe the stimuli. In the second condition, the same stimuli were presented, but the participants were instructed to lift the right finger in response to their presentation. The fundamental comparison was between trials in which the subjects performed the movement in response to an observed action (imitation) and trials in which the movement was triggered by the cross (a *non-imitative* behavior). Activation of the MNS was significantly stronger during imitation than during non-imitative behavior and passive observation of stimuli. These results were subsequently confirmed by Koski and coworkers (2002) and Grèzes and coworkers (2003).

It has been also proposed that the MNS is involved not only in imitative behaviors but in imitation learning. Buccino and coworkers (2004b) investigated the neural basis of imitation learning in an fMRI study, where participants who never played guitar before were asked to imitate guitar chords played by an expert guitarist. The analysis was carried out on four epochs: 1) observation of the chords made by the teacher, 2) pause, 3) execution of the observed chords, and 4) rest. During passive observation there was activation of IPL, PMv cortex, plus the pars opercularis of IFG. During pause, the same circuit as during observation was activated but, most interestingly, there was also blood flow increase in the dorsolateral prefrontal cortex (area 46) and in the anterior mesial cortex. The authors proposed a two-step processing in imitation learning: first, mirror activation of motor act representations in the parietal and frontal lobe; second, recombination, performed by the prefrontal lobe (area 46), of these motor acts, in order to fit the observed model. The same authors carried out a subsequent fMRI study in expert and naïve guitarists (Vogt et al. 2007). In this event-related fMRI study, they demonstrated the effects of a single session of practicing to configure hand actions (guitar chords) on cortical activations during observation, motor preparation and imitative execution. During the observation of non-practiced actions, the MNS was more strongly activated than for the practiced actions. These findings indicates a strong role of the MNS in the early stages of imitation learning. In addition, the left dorsolateral prefrontal cortex was selectively involved during observation and motor preparation of the non-practised chords.

Particularly interesting also from a clinical point of view (see next chapter) was the demonstration that the mirror mechanism is involved in the building of motor memories. The most convincing evidence for such a role came from TMS studies by Stefan and coworkers (2005; 2008). The authors showed that when participants simultaneously performed and observed congruent movements, the learning of these movements was potentiated with respect to learning through motor training alone. These findings indicate that the coupling of observation and execution strongly facilitates the formation of motor memories.

1.1.7 Action observation in human infants

Increasing evidence has recently been gathered in support of the notion that a mirror mechanisms matching action execution and action perception might be present during infancy and that their neural substrate might involve a network centered on central, frontal and parietal nodes (Shimada and Hiraki 2006; Nystrom 2008; Nystrom et al. 2011; Marshall et al. 2011; see for a review Burzi et al. 2015). The majority of these reports are based on EEG recordings, while a few others used near infrared spectroscopy (NIRS), a non-invasive technique allowing functional brain studies, which targets circumscribed brain regions. Thus, a precise mapping of the entire MNS in infants is missing (Shimada and Hiraki 2006; Grossman et al. 2013). The strongest evidence for a neural signature of action observation comes from studies exploring the desynchronization of the mu-rhythm, which was reported for both occluded and visible goal-directed grasp. Taken together, these studies suggest that, in early infancy, a direct visual–motor matching process is already detectable at as early as 6 months, suggesting a matching between action perception and execution already in infancy.

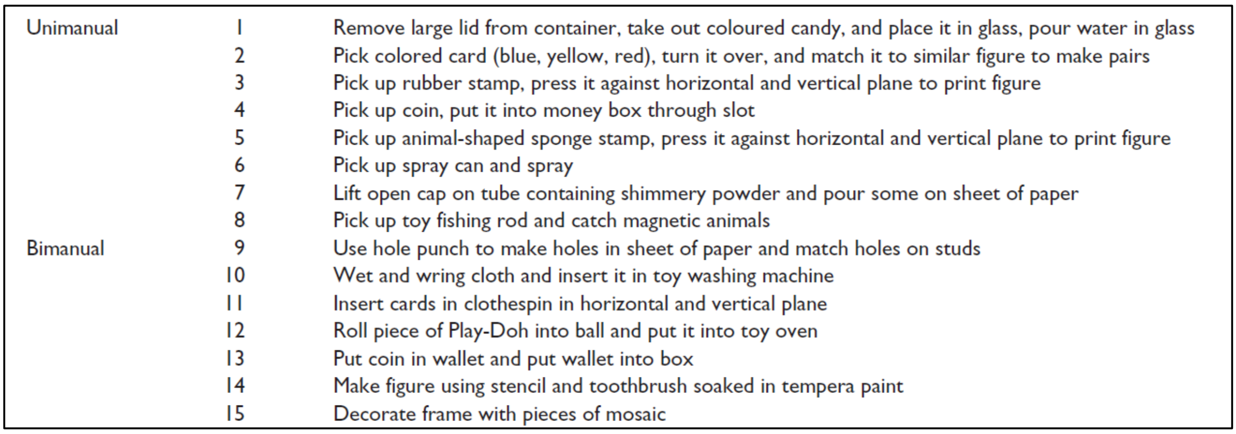
The problem of the age at which infant mu-rhythms respond to hand actions was addressed by Nystrom and coworkers (2008, 2011). They measured mu-desynchronization and ERP responses during observation of videos of both goal-directed and non-goal-directed motor actions. They found a significantly higher ERP amplitude for goal-directed grasp, while the differences in mu-desynchronization were not significant. The same authors proposed a similar experiment in 8-months-old infants, replacing the observation of video stimuli with live ones, demonstrating this time a significantly greater response to goal-directed actions also in the mu-desynchronization. Unfortunately, comparison of the two studies does not allow clarification of whether the effect on mu-desynchronization in the group of older infants is caused by brain maturation, type of stimulus (live versus video), or other sample-related biases. Most studies investigating action observation compared the effects of observing an action with rest condition or with the observation of neutral visual stimuli. Grasping of an object was the most commonly used action. However, the sensorimotor regions were found to react to several other types of actions including partly occluded grasps (Southgate et al. 2010), button press (Marshall et al. 2011), intransitive movements (van Elk et al. 2008; Nystrom et al. 2011; Reid et al. 2011; Warrein et al. 2013) or tool-executed grasps (Southgate et al. 2010).

Some neuroimaging studies have investigated the MNS in typically developing children or adolescents for different tasks (observation of hands grasping objects, Biagi et al. 2016; Ohnishi et al. 2004; observing face emotional expressions, Dapretto et al. 2006; imitation and observation of an animated finger, Williams et al. 2006; neutral and angry hand and face actions, Shaw et al. 2012). In a very recent fMRI study, Biagi and coworkers (2016) explored the activation of areas belonging to the MNS in children and adults during observation of complex hand-grasping actions, as compared to observation of simple grasping actions, executed with the left and the right hand, seen from a first-person perspective. The results indicate that during the action observation tasks in children there was an activation of a cortical network similar to that found in adults, including the premotor cortex, the posterior part of the IFG and the posterior parietal lobe. However, the activation in children was more widespread and showed a higher inter-subject variability when compared with adults. Furthermore, the activated network was more lateralized to the left hemisphere in adults and more bilateral in children, with a linear growth of lateralization index as a function of age. Finally, in children the activation in the AIP of each hemisphere was higher during observation of the contralateral hand (hand identity effect) and during the observation of complex actions relative to simple ones, confirming the role of AIP for action-related hand identity previously described in adults. Their results support the assumption that structure and size of action representations are sensitive to mechanisms of development and show physiological plasticity. In another work, Ohnishi and coworkers (2004) investigated with fMRI brain activation of 7–13-year-old children during observation of hand-grasping objects versus hand static presentation, founding activations in the PMd cortex, the parietal operculum, the IPS and the STS during objects-related hand actions. Similar results were found by Pokorny and coworkers (2015) investigating possible differences between adolescent with Autism Spectrum Disorders (ASD) and healthy adolescents during encoding of transitive and intransitive actions. Their findings showed that object presence modulated activity in the right IFG and supramarginal gyrus of the healthy children, while no modulation was seen in the ASD group. However, at a between group level there were no significant differences between the control and ASD groups. This suggests that there was no global deficit of the MNS in individuals with ASD while observing transitive and intransitive actions, but a different modulation during the observational conditions.

1.2 Mirror Neurons and upper-limb motor rehabilitation

1.2.1 The Action Observation Therapy in adults

Recently it has been proposed that the systematic use of action observation followed by imitation (AOT - Action Observation Therapy) is an effective way to enhance motor experience and promote upper-limb recovery in neurological and non-neurological patients (for review, see Garrison et al. 2010; Buccino 2014). Now AOT is considered a novel rehabilitation approach exploiting the mirror mechanism and its potential role in motor learning for motor recovery. Typically, during a session of AOT, daily actions chosen on the basis of their ecological value (e.g. grasping a key and inserting it into a whole) are practiced during a rehabilitation therapy that lasts about two-to-four weeks (3-5 days a week). For example, Table 1 lists some actions trained during AOT in children (Sgandurra et al. 2011; 2013). During each rehabilitation session, patients are required to observe a specific object-directed daily action presented through a video clip on a computer screen, and afterwards to imitate what they have observed. Usually only one action is practiced during each rehabilitation session and the action can be divided into three to four motor acts. For example, the action of drinking a cup of tea can be decomposed into the following motor acts: 1) pouring the tea, 2) adding sugar, 3) turning the spoon and finally 4) bringing the cup to the mouth.



Unimanual	1	Remove large lid from container, take out coloured candy, and place it in glass, pour water in glass
	2	Pick colored card (blue, yellow, red), turn it over, and match it to similar figure to make pairs
	3	Pick up rubber stamp, press it against horizontal and vertical plane to print figure
	4	Pick up coin, put it into money box through slot
	5	Pick up animal-shaped sponge stamp, press it against horizontal and vertical plane to print figure
	6	Pick up spray can and spray
	7	Lift open cap on tube containing shimmery powder and pour some on sheet of paper
	8	Pick up toy fishing rod and catch magnetic animals
Bimanual	9	Use hole punch to make holes in sheet of paper and match holes on studs
	10	Wet and wring cloth and insert it in toy washing machine
	11	Insert cards in clothespin in horizontal and vertical plane
	12	Roll piece of Play-Doh into ball and put it into toy oven
	13	Put coin in wallet and put wallet into box
	14	Make figure using stencil and toothbrush soaked in tempera paint
	15	Decorate frame with pieces of mosaic

Table 1: List of Goal-Directed Actions used for the AOT in children affected by Unilateral Cerebral Palsy (Sgandurra et al. 2011; 2013).

Each motor act is typically observed for about 3 min, so that the whole duration of a video sequence depicting a specific daily action is about 12 min (3 min x 4 motor acts). In the video, each motor act is performed by both a healthy actor and actress and is seen from different perspectives (frontal or lateral view). A consideration regarding the point of view from which the stimuli are presented comes from a study by Caggiano and coworkers (2011) in which the visuo-motor responses of the monkey MNS were recorded during the presentation of movies showing motor acts performed by others from different perspectives. The results showed that the majority of the tested mirror neurons (74%) exhibited view-dependent activity with responses tuned to specific points of view. A minority of the tested mirror neurons (26%) exhibited view-independent responses. The authors propose that view-independent mirror neurons encode action goals, irrespective of the details of the observed motor acts, whereas the view-dependent ones might contribute to a modulation of view-dependent representations in higher-level visual areas, potentially linking the goals of observed motor acts with their pictorial aspects.

The possibility to use an observation/execution approach in neurological patients has been exploited in several studies with hemiplegic adults after stroke (Ertelt et al. 2007; Celnik et al. 2008; Franceschini et al. 2010; Franceschini et al. 2012; Cowles et al. 2013; Sale et al. 2014; Sugg et al. 2015). In the first absolute clinical trial on AOT, Ertelt and coworkers (2007) enrolled 16 patients with moderate post-stroke hemiparesis who were randomly assigned to either the experimental or the control group. The experimental group performed an action observation training based on the combination of action observation followed by repeated motor training of the observed actions for 90 minutes per day with the paretic hand. Actions of increasing complexity were observed and imitated each day for 18 days. The control group performed the same upper limb actions of the experimental group on verbal instruction, while they observed only geometrical symbols and letters. Significant functional improvement on standard scales occurred for the experimental group compared with controls and was maintained at 8 weeks post-training. In addition, before and after training, participants of both groups performed an independent object manipulation task while scanned with fMRI. The data revealed, only in the experimental group, a significant increase in neural activity for motor-related brain regions including the MNS. More recently, Franceschini and coworkers (2012) performed a randomized controlled observer blind trial to evaluate the effectiveness of an AOT as an add-on treatment to the standard rehabilitation of upper limb, early after stroke. The results showed an improvement over time appreciated in all measures of motor impairment and functional ability for the experimental group. Moreover, a time x treatment interaction emerged from the analysis of motor performance at the Box and Block test, with a significant difference T0-T1 and T0-T2 in the experimental group compared to the control one.

In other randomized controlled trials, the effectiveness of the AOT has been investigated in patients with Parkinson Disease (PD) to complement pharmacology in the treatment of these patients (Pelosin et al. 2010; 2013; Buccino et al. 2011). In the first trial on PD patient by Buccino and coworkers (2011), patients of the experimental group observed videos depicting everyday life actions, including postural actions and walking, while participants of the control group observed videos without explicit motor content. Their findings showed that there was an improvement in the experimental group on two functional scales, the Unified Parkinson's Disease Rating Scale and the Functional Independence Measure (FIM). Moreover, AOT has been utilized successfully as application therapy in remediation of freezing of gait in PD patients (Pelosin et al. 2010).

More interestingly, recently it has been shown that AOT can be used for improving autonomy in ADL and balance in postsurgical orthopaedic subjects (hip fractures or hip or knee replacement) (Belelli et al. 2010) and to enhance knee joint function after total knee replacement (Park et al. 2014). For example, in the study of Bellelli and coworkers (2010) all participants underwent conventional physiotherapy. In addition, patients in the case group were asked to observe video clips showing daily actions and to imitate them afterward. Patients in the control group were asked to observe video clips with no motor content and to execute the same actions as patients in the case group. Participants were scored on functional scales (FIM and Tinetti scale) at baseline and after treatment by a physician blinded to group assignment. Their results showed that after treatment, patients in the case group scored better than patients in the control group.

1.2.2 The hemiplegic forms of cerebral palsy

Recently it has been proposed that action observation followed by immediate reproduction could represent a tool for motor recovery also in children affected by spastic cerebral palsy. This definition encompasses a heterogeneous group of neurodevelopmental conditions that present primarily as disorders of movement and posture, often accompanied by epilepsy, secondary musculoskeletal problems, and impaired sensation and cognition (Rosenbaum et al. 2007). Symptom onset occurs during early childhood, typically before 18 months of age (Rosenbaum et al. 2007); on average, diagnosis is confirmed at 13–19 months (Parkinson et al. 2010; Boyd et al. 2013). Cerebral palsy, by definition, results from abnormal brain development and/or brain damage that is non-progressive and occurs during very early development. In most cases, the cause is periventricular white matter damage that is presumed to occur during the third trimester of pregnancy, but other abnormalities, such as diffuse grey matter injury, focal infarcts, lesions of the basal ganglia, and/or cerebral malformations, can underlie the condition (Bax et al. 2006; Krägeloh-Mann and Horber 2007; Towsley et al. 2011). Early brain injury that can underlie cerebral palsy can lead to atypical brain development and reorganization, particularly during the first 2 years of life (Eyre et al. 2001) which can complicate the understanding of the condition and the selection of appropriate rehabilitation strategy (see next section: brain reorganization). The current definition of cerebral palsy includes the word *permanent*, but notes that “initial disruption to normal brain structure and function … may be associated with changing or additional manifestations over time” (Rosenbaum et al. 2007). In children with cerebral palsy, the type and extent of impairment is primarily determined by the location and size of the brain lesion (Holmefur et al. 2013). The ability of patients to gain functionality with therapy might be influenced by comorbidities, such as impaired vision and concentration, learning difficulties, and epilepsy (Novak et al. 2013).

The most common form is unilateral cerebral palsy (UCP) or hemiplegia, which impairs the use of one hand and consequently disrupts bimanual coordination. Children with UCP represent 39% of general cerebral palsy population (Shevell et al. 2009) and the second in terms of frequency, after diplegia, in premature infants (around 20% of cases) (Hagberg et al. 1996; Himmelman et al. 2005). Traditionally, hemiplegia is defined as a central unilateral palsy that only affects one side of the body, almost always of *spastic* type (Aicardi and Bax 2009), while the term *hemidystonia* is more adequately used to define the *dyskinesic* form. Compared to cerebral palsy, a distinction is made between a *congenital* form of hemiplegia, when the lesion occurs before the end of the neonatal period, and an *acquired* form, when the lesion provoking hemiparesis occurs later, within the first year of life. In many cases of hemiplegia, it is not possible to trace back, in the infant’s personal or family history, the etiopathogenic factors that determined the cerebral lesion. This can be confirmed for normal term infants, while for premature infants both pre – and – perinatal factors are frequently correlated to the lesion (Cioni et al. 1999).

The main clinical characteristic of hemiplegia is the *reduction of the motor repertoire* of the affected hemiside in module acquisition (the elementary components of movement that child is provided with), combinations (the capacity to subdivide the individual modules into different patterns according to space relations) and sequences (ability to assemble the individual modules according to different time relations). These early clinical signs allow to prompt a diagnosis of UCP (Ferrari and Cioni 2009). The clinical history of the hemiplegic forms of cerebral palsy testifies how early the motor repertoire is altered. Motor abnormal signs occur generally on both sides during the first weeks after damage, especially for premature infants with prenatal form, and can also further worsen with time. In particular, a further reduction of the residual motor repertoire, especially of the upper limb, is possible when new postural and displacement competences are organized (sitting position, upright standing position, horizontal locomotion, start of walking). After the first years of life, the probability that the patient's residual motor repertoire can be modified is scarce (concept of gate closure).

Another clinical sign that is often reported in hemiplegic cerebral palsy is the presence of *associated movements* that express the relation and mutual influence among the preserved hemiside, the plegic hemiside and the different segments of the plegic hemiside. They are traditionally classified into *synergies* (activation of a motor module at a distal level allows complete expression of the combination and sequence inside the limb) and *synkinesis* (involuntary movements produced by plegic hand when voluntary movements are made by the preserved hand). One of these particular components is imitation *synkinesis* or *mirror movements,* with opposite direction. These movements are also highly indicative of reorganization of hand control in the intact hemisphere, which then guides both hands.

1.2.3 Classifications of spastic unilateral cerebral palsy

A first classification of spastic UCP is based on neuroimaging techniques, especially MRI, that enabled very encouraging studies on the natural history of the lesion and the factors determining it. This type of classification subdivided UCP into malformation groups (cysts of different nature, schizencephaly etc.) periventricular lesions (leukomalacia), atrophy and dilatations of the lateral ventricles, especially at the level of the atria, cortico-subcortical lesions (porencephalic cysts, areas of perilesional gliosis), diencephalic lesions (affecting basal ganglia, thalamus, internal capsule) and diffuse lesions, as a result of infant cranial trauma. For examples, Krageloh-Mann and coworkers (2004, 2007, 2009) classified MRI results according to the etiopathogenic patterns in four groups:

1. Brain maldevelopments, or 1st and 2nd trimester patterns, presumed to occur in utero, such as lissencephaly, pachygyria, polymicrogyria, focal cortical dysplasia, accounted for 16% of the cases.

2. Periventricular white matter (PVM) lesions related to the early 3rd trimester of pregnancy and preterm infant, such as periventricular leukomalacia (PVL) defects following intraventricular haemorrhage (IVH) or periventricular haemorrhagic infarctions, accounted for 36%.

3. Cortical or deep grey-matter lesions that occur towards the end of gestational age, or late 3rd trimester patterns and peri-or-neonatally, like basal ganglia/thalamus lesions, parasagittal injury, multicistic encephalomalacia and middle cerebral artery infarcts, were noted in 31% of the cases.

4. Abnormal miscellaneous patterns not meeting the above criteria were seen in the rest of the cases.

Considering the criteria of the type and timing of the lesion, another classification of spastic UCP proposed by Cioni and coworkers (1999) identifies the following four typology of childhood hemiplegia (see Table 2):

- Type I (early malformative);
- Type II (prenatal);
- Type III (connatal);
- Type IV (acquired)

The specific characteristics of lesions belonging to the four groups are reported in Table 2. The main factors that determined both the clinical characteristics of hemiplegia with its natural history, and the possibility, modality and efficacy of post-lesional reorganization (see next section) are the type of lesion and its timing.

Other individual factors, like lesion size and protective genetic and/or environmental factors also play an important role in determining the variability of the clinical picture. The validity of Cioni and coworkers's classification (1999) has been confirmed in a larger hospital based population of 165 children with UCP (Petacchi 2008).

	Type I **MALFORMATIVE** Lesion of the 1st and 2nd trimester	**Type II** **PRENATAL** Lesion of the 3rd trimester	**Type III** **CONNATAL** Perinatal lesion at term	**Type VI** **ACQUIRED** Early acquired lesion
Lesion type	Often complex malformative cerebral pictures, especially related to early migration disorders (cortical dysplasia, schizoencephaly etc.) Sometimes encefloclastic cyst, mostly extened.	Haemorrhage of the PWM, mostly unilateral, or asymmetrical, resulting from periventricular leukomalacia; at MRI during chronic stage, frequent encefaloclastic cysts inside dilated lateral ventricles and possible gliotic areas also in contralateral PWM.	Frequent cortico-subcortical lesions due to the infarction of a major cerebral artery (mostly the main branch or one main cortical branch of the median cerebral artery). Sometimes lesions affect the deeper branches with involvement of the diencephalic structures (especially the upper arm of the internal capsule), thalamus and basal ganglia affection of the putamen.	Malacic and/or gliotic results mainly due to thrombotic occlusion of intracranial arteries in the area of median cerebral distribution (as results of trauma, infections, vascular malformations, or others).

Table 2: Classification of forms of hemiplegia in children according to brain lesion type and timing (Cioni et al. 1999).

In recent years, Cioni and coworkers (2009) have attempted to validate a recent classification of manipulation in children with spastic hemiplegia (see Table 3 for a description of main hand features related to classification). It describes five patterns of manipulation in hemiplegic children by analyzing hand kinematic profile and functional use (Cioni and Ferrari 2009).

Class	Integrated	Semi-functional	Synergic	Imprisoned	Excluded
Hand	Semi-opened with almost completely extended fingers.	Semi-opened with quite extended fingers.	Semi-opened with semi-extended metacarpo-phalangeal joints, semi-flexed and slightly abducted fingers.	Frequently closed fist enclosing thumb, or flexed wrist, semi-flexed fingers and adducted thumb (placed almost underneath palm).	Semi-opened with generally flexed wrist and semi-extended or more or less flexed fingers.
Thumb	Slightly abducted. Subterminal opposition with index and/or middle finger.	Aligned or almost abducted. Subterminal/terminal or lateral opposition with index and/or middle finger.	Adducted or sometimes positioned underneath other fingers, but never imprisoned.	Imprisoned in the palm between index and middle finger or between middle and ring finger.	Aligned or fairly abducted. Can be adducted but functionally not opposed.
Finger Movements	Possible selective finger movements, especially of index finger, with good variability.	Still possible selective finger movements, especially of index finger, but with reduced variability.	Only possible combined fingers movements. Possible independent index activity.	Impossible independent fingers movements, index autonomy is sometimes still present.	Very difficult independent finger movements, except for overall adduction.
Pinch or grasp	Subterminal/terminal bi – or tripodal distal pinch.	Subterminal/lateral bi – or tripodal distal pinch.	Still possible the tripodal pinch, but with poor thumb adaptation.	Indirect digit-palmar or interdigital grasping through passive placement of object by unaffected hand.	Inefficient or absent lateral thumb/index pinch.
Wrist	Rather extended and sufficiently mobile.	Slightly flexed but sufficiently mobile.	More or less semi flexed with an ulnar deviation. Generally not very mobile.	Flexed with an ulnar deviation. Active and passive stiffness.	In flexion. Passively mobile.
Forearm	Neutral or slightly pronated. Possible active supination.	Semi pronated with limited possible supination.	Semi pronated with reduced or completely absent supination.	Pronated with reduced or completely absent supination (also passive).	Semi pronated or in neutral position. Difficult or very limited active supination.
Elbow	Slightly flexed, generally mobile.	Semi flexed, generally mobile.	Semi flexed and still sufficiently mobile.	Frequently flexed with poor mobility.	Usually semi flexed, generally with acceptable mobility.
Arm	Aligned.	Slightly intra-rotated.	Slightly abducted and intra-rotated.	Abducted and intra-rotated.	Positioned near trunk.
Shoulder	Good general mobility in neutral pattern.	Mobile, only slightly lowered and antepulsed.	Mobile, slightly lowered and antepulsed.	Poor mobility, somewhat lowered and antepulsed.	Mobile, sometimes slightly lowered and antepulsed.

Table 3: Proposal of classification of manipulation patterns in hemiplegia (Cioni and Ferrari 2009). Continue in next page.

Grasping modality	Grasping of medium or small size objects through good hand adaptation. Grasping is not influenced by the activity of the unaffected hand.	Grasping of medium size objects through acceptable hand adaptation. Grasping is somewhat possible also if unaffected hand is performing another task.	Simple synergy of grasping triggered through controllable elbow and shoulder movements and under visual control. No hand adaptation to object. Grasping is possible only if unaffected hand takes part in the same action.	Indirect grasping (passive placement with thumb imprisoned or positioned underneath palm). Possibility: - Inter digital grasping or hook like with second and third finger; - Positioning of fist on flat surface in order to hold, push, press, hit, etc. - Bimanual grasping through opposition of the wrist against unaffected hand;	Neglect. Inefficient or absent grasping. Sometimes, after several attempts momentarily grasping of a thin and light object, through an inferior grasp between thumb and palm. Upon request, possibility to fix between flexed wrist and flat surface under constant visual control and without any hand adaptation.
Reaching	Orientation, anticipation, preadaptation. Motor pattern combinations without restriction.	Orientation, anticipation and pre-adaptation possible, but more uncertain. Restricted motor pattern combinations.	Orientation, anticipation and pre-adaptation with insufficient motor planning modulation. Reaching dependent on flexion and extension synergies.	Orientation without modulation. Impossible anticipation and pre-adaptation of the hand. Difficult reaching due to freezing of elbow and wrist.	Reaching very difficult or absent.
Visual-motor integration	The object can be spontaneously passed from one hand to the other without visual control.	The object can still be passed from one hand to the other. Necessity of non-continuous visual control.	The object is passed with difficulties from the affected hand to unaffected one, better vice versa. Necessity of visual control.	The object is held in the affected hand through the placement by the unaffected one (passive loading). Necessity of constant visual control.	Usually there is no visual attention towards the hand, which is often outside visual field. The object can be picked up by the affected hand only after insistent request and with continuous visual control.
Releasing	The object is readily released, without difficulty, without necessity of visual control.	The object is readily but roughly released. Necessity of frequent visual control.	The object is released slowly and with difficulty, often using servomotor movements.	Difficult hand opening. The object is released only with great difficulty. Generally the unaffected hand pulls it out.	Poor capacity to hold the object in the hand, frequently unintentionally dropping it. Object is preferably pulled out rather than voluntarily released by hand opening. In any case poor timing of release.
Manipulation	Mastery of intrinsic motricity for manual exploration.	Scarce or absent intrinsic motricity. The hand still adapts to the object, but performs complex movements with difficulty.	Extremely limited. The manipulation is possible only if the object possesses suitable dimension, weight and consistency.	No manipulation of object is possible.	Impossible.
Bimanual activity	Good collaboration between hands also for complex manual activities. There is a discreet part of lateral hemispace in which the affected hand can be spontaneously used first.	Good cooperation between the two hands. Positioning of affected hand near trunk during complex activities. There is only an extreme part of lateral hemispace in which the affected hand can be spontaneously utilised first.	Possible collaboration in achieving the same aim. Affected hand is used to support the unaffected one. There is no part of lateral hemispace in which the affected hand can be spontaneously utilised first.	Cooperation, if strictly necessary through passive loading of the affected hand. Possibility to load the affected hand only in the immediate and paramedian space. Use of alternative grasping solution.	Hyper specialization of unaffected hand and absence of bimanual activity. Use of the affected hand only upon insistent and explicit external request. Use of alternative grasping solutions.
Main core	Subterminal/terminal pinch. Intrinsic motricity.	Subterminal/lateral pinch with basically adducted thumb.	Stereotypically expressed grasping and releasing within flexion and extension synergies. Active loading of object.	Indirect grasping (passive loading). Imprisoned thumb or positioned underneath palm.	Functionally ineffective or negligible grasping.

1.2.4 Reorganization of the sensorimotor system after early brain damage

Over the years, animal data have demonstrated that the effect of a lesion of the developing brain depends on the point in time at which the lesion occurred. Originally, it was thought that “the younger the age at insult, the better the outcome” (the so-called Kennard-principle; see Kennard 1936). But gradually it became clear that this is not always true (Kolb et al. 2013). Many factors determine the consequences of a lesion of the developing brain: the age at insult, the site and the size of the lesion, its unilateral or bilateral nature, animal species, sex, exposure to chemical substances prior to and after the insult, and environmentally induced experience. Rodent studies indicated that, in particular, two types of environmental experience are associated with improved outcome: being raised in a complex environment and tactile stimulation at early age (Kolb et al. 2011, 2013). Animals with an early lesion of the brain who are raised in a complex environment, including attractive toys and peers, have a significantly better motor and cognitive outcome than lesioned animals brought up in a standard laboratory environment. The complex picture emerging from the animal studies is that, each age, each neural system, each species, and each sex have specific vulnerabilities and resources of resilience to cope with the effects of an early lesion. Nevertheless, within this complexity three general principles may be distinguished: (a) bilateral lesions are associated with a lower potential for functional plasticity and with worse outcome than unilateral lesions; (b) large (unilateral) lesions are associated with less recovery and worse functional outcome than small (unilateral) lesions; (c) cognitive functions show a better recovery than motor functions (Kolb et al. 2013).

A recent review of population-based studies carried out in western industrialized countries, revealed that a lesion of the periventricular white matter is present in 19–45% of children with cerebral palsy (Reid et al. 2014). Other relatively frequent lesions are gray matter injury, including lesions of the cortical gray matter, the basal ganglia, and the thalamus (21%), malformations (11%), and focal cortical infarcts (10%). Note that in about 15% of children with cerebral palsy, structural MRI scans do not show abnormalities (Krägeloh-Mann and Horber 2007; Korzeniewski et al. 2008; Reid et al. 2014). Furthermore, these findings do not inform us about the neural mechanisms operating when the brain acquires a specific lesion at a certain early age. These mechanisms may involve plastic, restorative adaptations, but they also may result in deleterious changes.

Lesions of the periventricular white matter mostly originate between the 24th and 34th weeks of gestational age. Prospective imaging studies on the developmental sequel of damage of the periventricular white matter indicated that focal necrotic lesions (periventricular leukomalacia – PVL) are associated with a high risk for cerebral palsy (>80%, see Fazzi et al. 1994). The risk for cerebral palsy is higher in posterior than in anterior lesions (Rutherford et al. 2010). In addition, the severity of cerebral palsy following PVL depends on the severity of the cystic lesion: focal cysts generally give rise to bilateral cerebral palsy with a diplegic distribution and more extensive cysts result in bilateral cerebral palsy with a quadriplegic distribution (Rutherford et al. 2010). In fact, the cystic lesions are the tip of the iceberg of the pathology in the periventricular white matter, as the cystic lesions are surrounded by diffuse astrogliosis and microgliosis in the white matter (Kinney et al. 2012).

Another categorization of damage includes unilateral lesions of the brain. Basically, two types of unilateral lesions can be distinguished: (a) unilateral periventricular haemorrhagic infarction, occurring in preterm infants of 24–34weeks of gestational age, and involving the periventricular white matter ("preterm" lesions; Maitre et al. 2009; Staudt 2010) and (b) focal cortical–subcortical infarction, occurring around term age, and usually affecting the area of the medial cerebral artery ("term" lesions). The term lesion in general does not involve the periventricular white matter (Staudt 2010).

Evidence suggests that for infants with perinatal brain lesions, important phases of sensorimotor reorganization occur during the first year of life due to greater neuroplasticity in the early stages of brain development but, for the same reason, maladaptive forms of sensorimotor reorganization can also occur during this same time period (Shimada and Hiraki 2006). Normally, in humans, corticospinal projections develop transient ipsilateral projections early in development that are predominantly eliminated when maturity is reached (Eyre et al. 2001, 2007). In neonates, for example, focal TMS of the motor cortex evokes ipsilateral responses with similar threshold and amplitude as those obtained for contralateral responses; this indicates a bilateral innervation of the spinal cord from motor areas of both hemispheres (Eyre 2007). Longitudinal studies on healthy infants show a consistent withdrawal of ipsilateral corticospinal projections, while the contralateral projections are enhanced (Eyre et al. 2001; 2007). Functional and anatomical evidence in animal models and in humans demonstrated that such process depends also on the activity of environment and of experience (Martin et al. 2004, 2005; Friel and Martin 2007).

In congenital brain damage leading to cerebral palsy, different types of brain reorganization can be observed (Figure 7). The main mechanism for a reconnection of the brain to spinal cord is a reorganization that occurs within the ipsilesional cortex, in regions inside the primary motor cortex (M1) or non-primary motor areas (Boyeson et al. 1994; Donoghue et al. 1996; Hallet et al. 2001) (Figure 7 b).

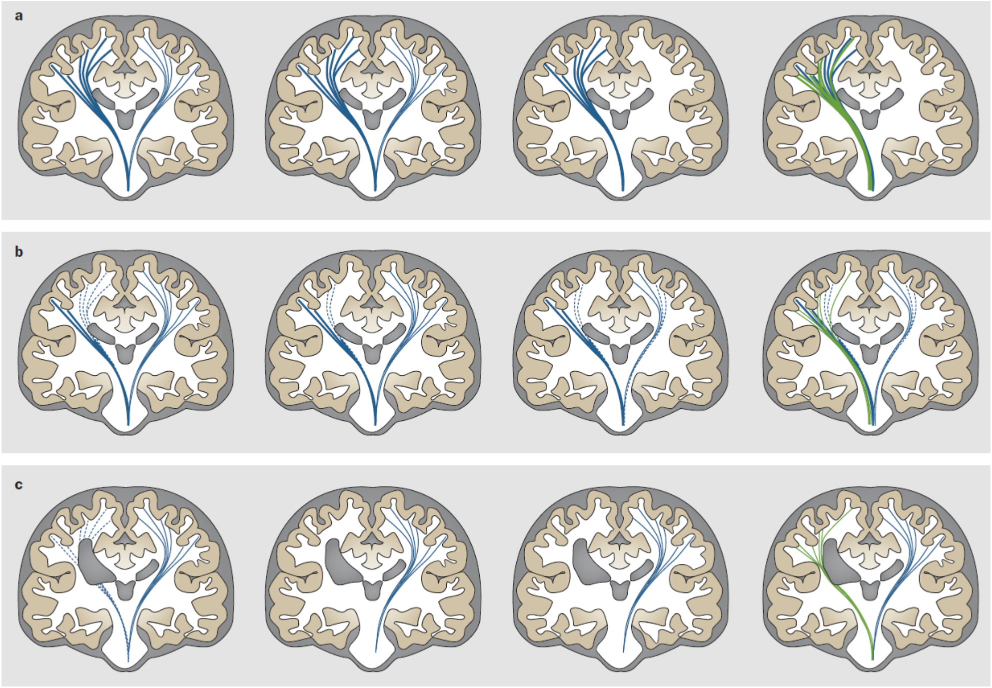


Figure 7: The influence of periventricular lesions on corticospinal laterality. Images show corticospinal (blue) and thalamo-cortical (green) connection pathways. **a)** In typical development, bilateral corticospinal connections develop and are unperturbed during early development. After birth, interhemispheric competition mediated by cross-callosal connections (not shown) results in the removal of connections that project to the ipsilateral side of the body. **b)** During early development in individuals with cerebral palsy who have small periventricular lesions, the lesions can weaken or eliminate the corticospinal connections, which are consequently unable to compete effectively with the opposite side of the brain during later development. The result is bilateral motor cortex control of the impaired side of the body. **c)** During early development in individuals with cerebral palsy who have focal or large lesions, the lesions might completely eliminate corticospinal connections in one hemisphere. (Modified from Reid et al. 2015).

In other cases, when the lesion occurs at an earlier stage of life, either during intrauterine life or soon after birth, a different mechanism can also be observed (Carr et al. 1993; Staudt et al. 2002, 2004; Guzzetta et al. 2007), in which a significant number of monosynaptic fast conducting ipsilateral projections from the undamaged cortex persists (contralesional reorganization; Figure 7 c). This mechanism allows the undamaged cortex to control directly both upper limbs (Staudt et al. 2004; Guzzetta et al. 2007). Staudt and coworkers (2002, 2007) detected a strikingly clear relationship between the extent of the periventricular lesion and type of motor reorganization: patients with small lesions showed a preserved ipsilesional projections to the paretic hand, whereas a majority of patients with large lesions have fast conducting ipsilateral pathways originating in the contralesional hemisphere.

1.2.5 Currently available rehabilitation strategy and AOT

Several models of intervention are currently available to improve upper limb function in UCP patients, such as intramuscular injections of botulinum toxin A (BoNT-A), alone or combined with upper limb training, “classic” constraint-induced movement therapy (cCIMT), hand-arm intensive bimanual training (BIM) and neurodevelopmental treatment (NDT) (Sakzewski et al. 2009). cCIMT and BIM are the two contemporary primary upper limb motor rehabilitative schemes increasingly used for children with UCP. CIMT was originally designed to address *learned disuse* of the impaired hand after stroke in adults, but has since been adapted to aid the development of motor skill in the impaired hand of children with UCP (Taub et al. 2004). A child-friendly protocol, called modified CIMT, involves constraint of the more-functional upper limb for several hours per day over several days. During this period, the impaired arm is trained in an intensive and repetitive manner in activity-based practice (Charles et al. 2006).

Patients undergoing BIM intensively practice bimanual tasks that become progressively more difficult. This therapy is based on the premise that co-ordination of both hands is important for the improvement of performance in everyday tasks, as these tasks are predominantly bimanual in nature. BIM focuses on tasks that require the use of both hands, as the unimpaired hand acts as a template for learning motor control of the impaired hand (Gordon 2011; Gordon et al. 2007).

Robot-assisted therapy is an emerging modality for rehabilitation that uses robotics to aid and/or objectively record movement of limbs during repetitive exercises. Robot-assisted therapies can allow participants with moderate impairment to experience a wider range of motion (and thus sensory feedback) than other therapies, and can be linked to virtual reality environments that provide haptic feedback. The technique is in the very early experimental phase and is primarily used in adults after stroke; its potential for therapy in UCP is currently unclear (Lo et al. 2010; Gilliaux et al. 2015).

The efficacy of the current activity-based therapies, as determined by large systematic reviews and meta-analyses, can be used to gauge the optimal dose, environmental context and intensity of upper limb intervention for children with UCP (Sakzewski et al. 2011; 2014). A meta-analysis of 42 randomized controlled trials assessed the efficacy of 14 approaches to upper limb therapy, and found moderate to strong evidence to support the use of intensive activity-based, goal-directed interventions (such as cCIMT and BIM) rather than usual care to improve the quality and efficiency of upper limb movement and to achieve individual goals (Sakzewski et al. 2014). The conclusions drawn in this systematic review, in combination with knowledge of the factors that impede or enable implementation of therapy, have led to the current consensus on the essential elements of effective upper limb therapy. These elements are intensive structured task repetition, progressive incremental increase in difficulty, and goal-directed approaches that enhance the motivation and engagement of individuals receiving therapy (Sakzewski et al. 2014).

Recently, AOT has also been used in children with UCP as a tool for motor rehabilitation. Sgandurra and co-workers (2013) enrolled two groups of homogeneous UCP children with a mild-to-moderate hand impairment; the experimental group observed video sequences of unimanual or bimanual goal-directed actions and subsequently executed the observed actions with the paretic limb or with both limbs, respectively, while the control group performed the same actions, after observing videos devoid of any action content. After training, the experimental group showed at the Assisting Hand Assessment (AHA) a greater improvement than the control one. Buccino and co-workers (2012) used a similar rehabilitation paradigm in children with different types of CP, showing a higher functional score, as assessed by the Melbourne Assessment Scale (MAS), in the case group than in the control one.

It has been proposed that in AOT the observation may elicit in the observer a marked propensity to preserve task proficiency, by selecting movements that guarantee to reach the action goal (see action goal representation in MNS areas), regardless of the kinematic resemblance of the observed model. This could be due to the fact that the complexity of models offered during observation of an action performed by a normal hand is not appropriate for imitation, so that UCP children decide to copy the final outcome of the action rather than the performance used to achieve it. This could also explain why UCP children do not simply imitate their unaffected hand.

Aims of the Thesis and Hypothesis

The main aim of this thesis is to highlight the role of motor experience in the modulation of the MNS in both healthy individuals and children with unilateral cerebral palsy. We hypothesize that the MNS could be better activated: 1) in healthy individuals by the observation of actions performed by a model with a motor repertoire similar to that of the observers; 2) in UCP children by the observation of a paretic hand model, kinematically similar to the observing child, as compared to a healthy one.

Throughout the last years, it has been demonstrated that changes in motor experience following intensive training in healthy individuals modify the brain response of the MNS/AON areas while observing skilled actions performed by experts. Starting from this assumption, several studies on action observation and motor expertise explored how specifically the practice of a certain activity could modulate the response of these areas during the observation of the same activity, like sports, dancing, playing piano. Most of the previous contributes investigated the effect of motor experience in population formed by elite skilled individuals, specialized in areas of expertise like archery, badminton, basketball, football (Aglioti et al. 2008; Abreu et al. 2012). Typically, these investigations compare the BOLD response during fMRI between groups of expert versus novice participants. Moreover, they used videos displaying actions performed by expert actors in that sport or novices that try to perform the same action with difficulty, often making mistakes in its execution. Overall, even if these studies used different paradigms and comparisons between experimental conditions, they seem to indicate that experts show a strong motor resonance with actions performed by the skilled model, not limited to the main areas of the MNS but extending to other brain regions. However, the interpretation of these results is often debated, because experts in a particular field have also a great visual familiarity with the observed action, in addition to the extended practice following intensive training.

Here, in the first study, we explore brain activations in a group of healthy individuals without particular hand motor skills observing complex manipulative actions. There are several differences with regard to the previous investigations in this field: 1) we did not compare groups of participants with different expertise, but enrolled healthy volunteers and assessed their manual skills ability, in order to have an homogeneous group in term of motor experience, hobbies and amusement; 2) we presented participants with complex object manipulation, performed with a right hand, in a first-person perspective; 3) all actions were performed with three different levels of difficulties, by three actors; 4) in the three conditions the content of the actions was the same, in term of goal and objects used, and all participants did not have previous experience with object manipulation in terms of visual familiarity.

The first hypothesis was that the MNS could be strongly activated in healthy individuals during the observation of manipulative actions that the participant can perform, such as finger/hand movements included in daily life actions. On the contrary, complex finger movements required during object manipulation could be less able to activate the MNS during observation, because they reproduce motor schema that are not present in the observer's motor repertoire.

The second hypothesis was that, even if a group of healthy individuals is homogeneous in terms of motor skill, it could be different in terms of small deviations in hand motor skills. This does not qualify the subjects' performance as "expert" or "naïve" (all-or-nothing) but identify slightly different levels of expertise, that could potentially influence action perception, as during observation.

The third hypothesis to test was that brain regions belonging to the MNS/AON, that are anatomically interconnected, are also functionally coupled during the observation of actions executed with a level of expertise similar to that of the observer. This modulation of the MNS was expected in particular within the main nodes of the parieto-premotor circuit, even if there may be differences between the functional role of separated streams in the action observation/execution network.

In a similar way, in the second fMRI study reported in this thesis, we tested the hypothesis that the MNS could be strongly activated also in children affected by UCP. Up to date, there are only few studies investigating the brain activation during action observation and execution in children with hemiplegia. For this reason, a first aim of this study was to explore the brain activations induced by action observation in a homogeneous group of UCP children compared with that of a group of typically developing children, matched by age. Previous studies on action observation in developmental age using neuroimaging techniques, reported that the MNS is more lateralized in adults then in children (Biagi et al. 2016), suggesting that despite the presence of extended unilateral lesions, the parieto-premotor circuit could be activated by observation of actions performed by another individual or by action execution. Moreover, a second important goal of this study was to investigate if in hemiplegic children the MNS is more strongly activated during the observation of actions performed by another hemiplegic child, with similar degree of hand impairment (pathological model), compared to actions performed by a child without motor deficits (healthy model).

The first hypothesis was that the MNS could be active not only in typically developing children, but also in hemiplegic children, despite the presence of extended lesions. In fact, different types of sensorimotor reorganization can be observed in hemiplegia, leading to different pathways of development, associated with a functional response of the MNS during action observation/execution.

The second hypothesis was that the MNS is more strongly activated in UCP children during the observation of actions performed by a pathological model compared to a healthy one, because it corresponds to a similar motor schema already embodied in the hemiplegic child’s own motor repertoire.

The results of this thesis, in a translational medicine perspective, could be very important for an observation-based therapy, because they allow to disentangle the brain mechanism underlying the processing of different aspects of action perception. Moreover, the results could allow to personalize the therapy in order to adapt it to the individual own motor repertoire with the goal to improve motor control of the upper-limb in hemiplegic children and other patients with acquired neurological disorders.

Study 1

Beyond the motor repertoire: neural mechanisms underlying observation of complex hand-object manipulation.

2.1 Introduction

It is now a well-accepted notion in neurophysiology that the observation of actions performed by others activates in the perceiver the same neural structures responsible for the actual execution of those same actions (Rizzolatti et al. 2014). Indeed, in the monkey, action observation is believed to activate the mirror neuron system (MNS), formed by visuomotor neurons which fire both when individuals perform a goal-directed action or when they observe the same, or a similar action, performed by another individual (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996). A comparable MNS has been identified also in humans using several electrophysiological and neuroimaging techniques (Buccino et al. 2001; Grafton et al. 1996; Grezes et al. 1999; Iacoboni et al. 1999; Caspers et al. 2010; Molenberghs et al. 2012). Its two main nodes are the inferior parietal lobule and the ventral premotor cortex, plus the caudal part of the inferior frontal gyrus (Rizzolatti et al. 2014; Rizzolatti and Fogassi 2014). Cortical brain areas in humans that have been shown to contain mirror neurons are often described as part of an action observation network (AON). The function of this network is to transform the sensory representations of observed motor acts into the corresponding motor representations, enabling the observers to automatically understand the goal of these acts. Overall, action observation is considered an effective tool to learn or enhance the performance of a specific motor skill in healthy people (Buccino et al. 2004; Stefan et al. 2005; Cross et al. 2006; Vogt et al. 2007; Cross et al. 2009; Sakreida et al. 2017). In fact, action observation has been shown to facilitate motor learning and the formation of a motor memory trace in normal adults (Stefan et al. 2005), and in clinical populations, such as patients suffering of ischemic stroke (Ertelt et al. 2007; Celnik et al. 2008; Franceschini et al. 2010; Buccino 2014). Another important assumption concerns the plasticity of the MNS. Several studies (Buccino et al., 2004; Calvo-Merino et al., 2005; 2006; Cross et al., 2006) indicate a strong role of the MNS in representing previously acquired motor skills, showing that, during action observation, observers tend to “resonate” more strongly with actions already embodied in their own motor repertoire. Starting from these assumptions, most studies on expertise investigated how the acquisition of a skilled action, such as sport or dance moves, affects AON activity while observing the movements involved in these actions (Buccino et al. 2004a; Calvo-Merino et al. 2005, 2006; Wright et al. 2010, 2011; Abreu et al. 2012). Typically, these studies compared the blood-oxygen-level-dependent (BOLD) fMRI response between experts and novices. Fast ball sports, like tennis or volleyball, provide perfect tasks to investigate the process underlying the anticipation of action effects as well as the influence of the athlete’s prior perceptual and motor experience (Aglioti et al. 2008; Abreu et al. 2012). Together, these studies suggest a strong link between action experience and motor resonance during action observation. The question arises if the same principle may be applied to the observation of novel hand manipulative actions beyond the personal motor repertoire, in terms of motor experience with the observed action. In this field, few neuroimaging studies compared directly the modulation of the parieto-frontal MNS in response to observation of complex versus simple hand-object manipulative actions (Binkofski et al. 1999; Gazzola et al. 2007; Biagi et al. 2010). However, to the best of our knowledge, no studies have investigate the influence of the own level of hand motor dexterity on the activity of the MNS. Moreover, previous studies considered the motor resonance an all-or-nothing concept. Thus, it is unclear if different levels of motor expertise can be associated to progressively different levels of motor resonance during action observation.

The aim of this study was to investigate a possible modulation caused by the observation of different levels of hand/fingers motor dexterity on the MNS activity. The hypothesis is that the MNS is more active when the observed hand actions are endowed within the own motor expertise, while the same circuit may be less active during the processing of hand actions that are beyond the own motor repertoire. Moreover, a second aim of this fMRI study was to investigate the modulation induced by the task (observe different levels of expertise) on the functional connectivity pattern between brain areas involved in the processing of complex object-related manipulative actions.

2.2 Materials and Methods

2.2.1 Participants

Eighteen healthy volunteers (10 females; mean age 22.5 years ranging 18-25 years) with normal or corrected to normal vision, no history of neurological, orthopedic or rheumatologic disorders, and no drug or alcohol abuse were recruited. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects with particular manual ability (e.g., musicians, athletes, typewriting, etc.) were not included in the study. All participants were selected on the basis of their daily activities, in terms of hobbies and amusements. The study was approved by the local ethics committee (Parma) and participants gave their informed written consent in accordance with the Declaration of Helsinki.

2.2.2 Hand motor skills Assessment

Hand/fingers motor skills were tested in all participants on the same day of fMRI acquisition, using the Purdue Pegboard Test (PPT - Tiffin and Asher, 1948). It consists of a board with two parallel rows with 25 holes per row into which cylindrical metal pins have to be placed by the participant. After explanation, as well as demonstration of the task and three practice trials, participants were asked to place as many pins into the holes of the perforated board as possible within 30-s of each trial. Three trials were administered in two conditions, that is with the dominant (right) and the non-dominant hand; manual ability scores (PPT scores) were obtained by averaging the number of pins correctly placed during each condition (we consider for the successive analysis only the right-dominant hand PPT score). The PPT score was used in the subsequent fMRI regression analysis (see below) to test for a possible relationship between the degree of individual hand motor dexterity and brain activity associated with the observation of complex hand-object manipulative actions performed by a skilled model. Individual normalized PPT scores, mean and standard deviation are reported in Supplementary Material.

2.2.3 Experimental Design, Stimuli and Task

The experimental stimuli consisted of video clips displaying hand-object manipulation performed with the right hand, lasting 3-s each (see Figure 8 A). All the actions consisted in complex objects manipulation, presented in three different experimental conditions: 1) actions performed by an expert actor with a high level of manual/fingers dexterity (action observation Expert, *AO Expert*); 2) actions performed by an actor with an intermediate level of manual dexterity (action observation intermediate, *AO Intermediate*), after a 2 months training for improving his manipulation skills; 3) actions performed by a naïve subject, without particular experience in object manipulation or manual skills (action observation novice, *AO Novice*).

Actors hand motor performance was assessed using the PPT test, the Maximum finger tapping frequency and the Minnesota Dexterity Test (Jurgensen, 1943). Individual scores related to actor's performances are reported in Supplementary Material. Movies were recorded using a digital HD Camera and edited using AdobePremiere (www.adobe.com).

The actions were presented in a first-person perspective, in order to maximize the motor resonance (see Caggiano et al. 2011). The object used for manipulation was a ball, a cylinder or a coin. Each action was recorded twice to take into account the variability of the execution performance. As control condition, 3-s videos showing sequences of random fingers movements performed with the index, middle, ring or little finger (right hand) were used in order to control for the mere general processing of biological motion.

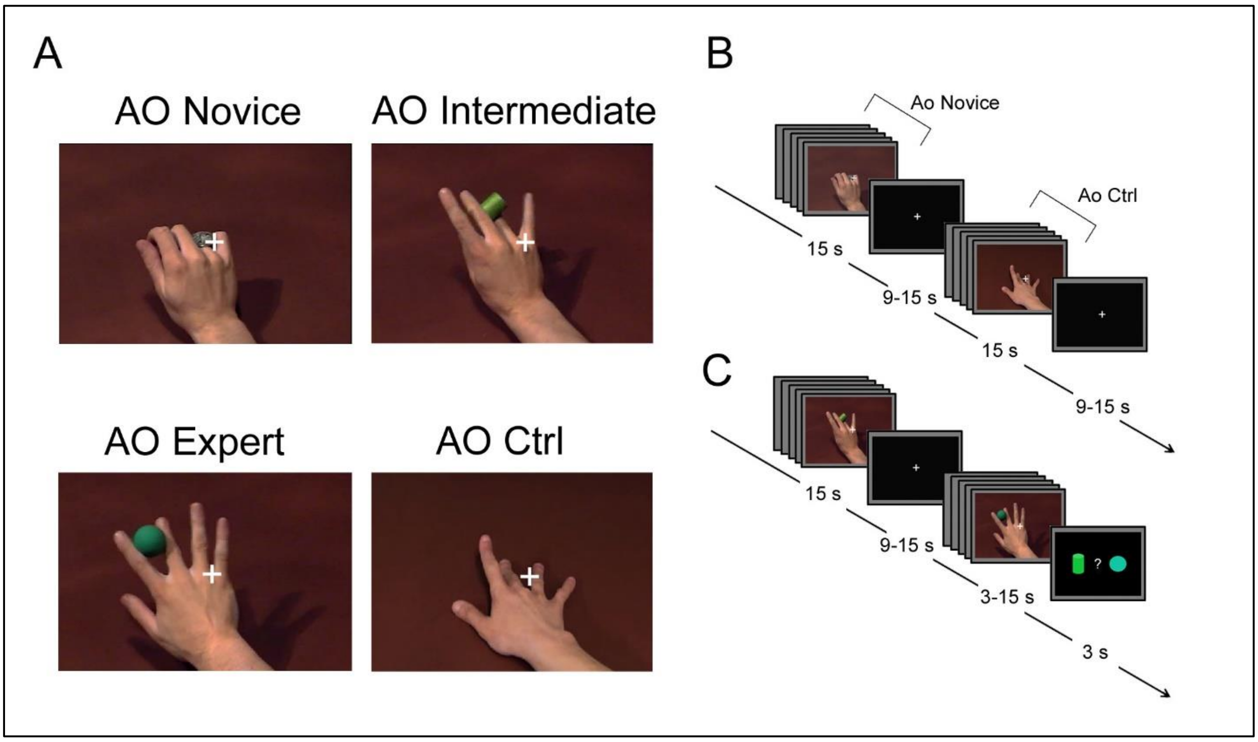


Figure 8: Experimental stimuli and task design. **(A)** In the action observation task, participants observed videos showing actions performed by a model without particular hand skills (AO Novice), by a model with intermediate level of hand ability after 2-month training (AO Intermediate) or by an expert model with high level of manual dexterity (AO Expert), from a first person perspective; actions consisted in hand-object manipulation performed with the right hand. In the control condition, participants observed simple extension-flection finger movements (AO Ctrl). **(B)** Action observation task was presented in two functional runs, each made up of 15-s independent blocks, consisting of five randomly presented videos of the same condition. Each block was interleaved by a *Rest* period (12-16-s). **C)** In 25% of action observation blocks, participants had to indicate the shape of the presented object.

For this condition we used sequences of fingers movements consisted in four consecutive extension-flexion movements (action observation Control, *AO Ctrl*). A total of 18 experimental video stimuli (3 actors x 3 objects x 2 repetitions) and 18 control stimuli were presented in the action observation task.

Motion Energy Quantification

Since video clips related to the main experimental conditions *AO Novice*, *AO Intermediate* and *AO Expert* varied in the amount of finger motion and velocity profiles (intrinsic features of the category of expertise) it was necessary to control that differences observed in brain activation were not due to low-level visual features. To take into account the amount of visual information between categories, we quantified the motion energy in each video clip using the Matlab algorithm VIP Motion working on Simulink. Such quantification of motion energy use motion detecting method. Similar methods were previously used in neuroimaging studies on action observation (Schippers et al. 2010; Cross et al. 2012). We calculated the sum of absolute differences (SAD) between each frame of each video clip for category, to measure the similarity between block of frames-images. SAD was calculated by taking the absolute difference between each pixel in the original block and the corresponding pixel in the block being used for comparison. These differences were summed to create a simple metric of block similarity. Results from the motion energy quantification procedure are illustrated in the Figure 9.

Action observation

Participants performed two functional runs after a short training session. They were instructed to passively observe all the actions presented paying attention to it. Experimental and control conditions were presented in independent 15-s blocks (see Fig. 8 B), constituted by five randomly presented videos (3-s each). Each run was arranged to include a total number of 16 blocks, 4 blocks for each condition. Blocks of stimuli were interleaved by a fixation–no clip event (*rest*) lasting 9-15-s, used as baseline. In 25% of the blocks, participants had to provide an explicit response (catch trials), using a response pad placed inside the scanner, concerning the shape of the object observed in the last video clip, thus unrelated to motor aspects of the tasks (Figure 8 C). The catch trials were randomly presented and lasted 3-s, followed by a 12-s rest period to remove artefacts derived from the motor component.

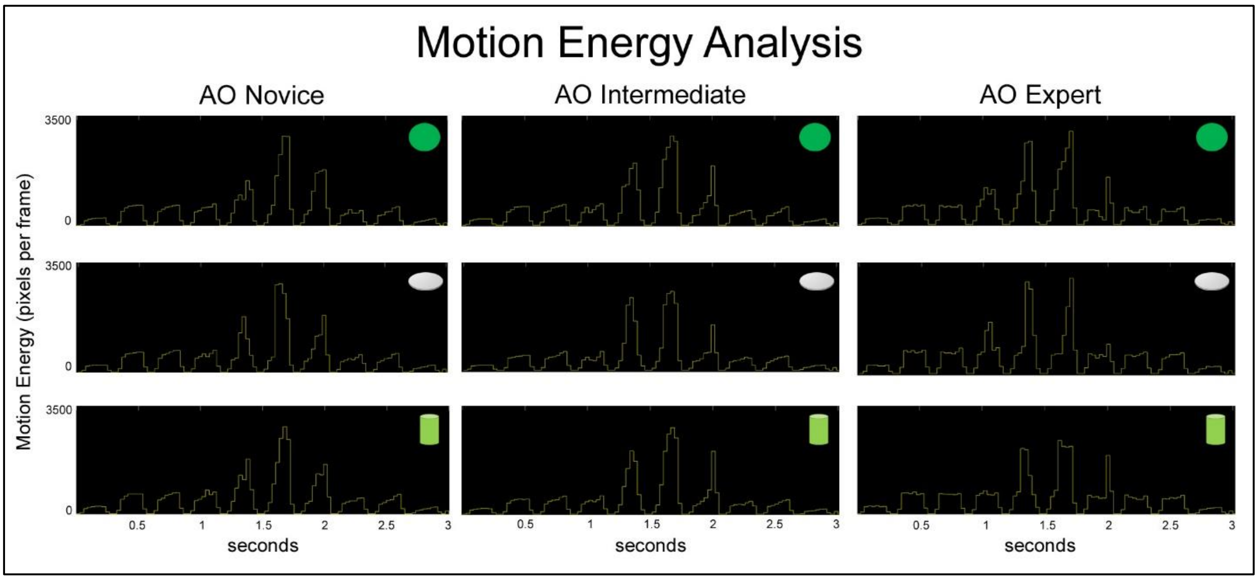


Figure 9: Time-course of motion energy quantification calculated as mean difference in pixels luminance between each pair of consecutive frames in each video. In each column are reported the results for each specific experimental condition. Rows correspond to motion energy analysis for actions performed with sphere (top row), the coin (middle row) and the cylinder (bottom row).

Action Execution Localizer

In a separate run subjects performed a Localizer task in order to define classical parieto-frontal areas involved in hand-object action execution. Before scanning, subjects were shown with the table (40 x 30 cm) that would be placed on his/her lap during scanning. The table contained a square box with a small cylinder inside. During task, participant was instructed to really perform object manipulation with his/her right hand. Each action started from the same starting position and terminated in the same final position. The task was performed in blocks lasting 15-s each, repeated 5 times. The task sequence was as follows. At the beginning of each block, an instruction cue (orange fixation cross) was presented for 2-s on the black screen, in order to control for movement preparation (action preparation, *Act Pre*). After 2-s, fixation cross color turned to green instructing subject to perform object manipulation (action execution, *Act Exe*). The baseline condition (*rest*) consisted of the static presentation of a white cross in the middle of the screen. The task was performed alternating experimental blocks and baseline period in a counterbalanced manner.

2.2.4 Scanning procedure

Participants went through a training phase before fMRI aimed at familiarizing them with the experimental procedure. They laid supine in the bore of the scanner in a dimly lit environment. Visual stimuli were presented in the fronto-parallel plane by means of a digital video system (60 Hz refresh rate) with a resolution of 800 horizontal pixels x 600 vertical pixels with horizontal eye field of 30° (Resonance Technology, Northridge, CA). Sound-attenuating (30 dB) headphones were used to muffle scanner noise and give instructions to subjects. Digital transmission of signal to scanner was via optic fiber. Software E-Prime 2 Professional (Psychology Software Tools, Inc., http://www.pstnet.com) was used both for stimulus presentation and recording of participant response. Before beginning of MRI acquisition, subjects received the instruction not to make any voluntary movement and only concentrate on the video screen. Absence of actual hand movement during tasks was visually checked by the investigator. Each of the action observation runs lasted about 8-min. The action execution run lasted about 5-min.

2.2.5 Data acquisition

Anatomical T1-weighted and functional T2*-weighted MR images were acquired with a 3-T General Electric scanner equipped with an 8-channel receiver head-coil. A three-dimensional (3D) high-resolution T1-weighted IR-prepared fast SPGR (Bravo) image covering the entire brain was acquired in one of the scanning sessions and used for anatomical reference. Its acquisition parameters were as follows: 196 slices, 280×280 matrix with a spatial resolution of 1×1×1 mm, TR = 9700 ms, TE = 4 ms, FOV = 252 x 252 mm; flip angle = 9°. Functional volumes were acquired while participants performed the action observation task and the action execution Localizer with the following parameters: thirty-seven axial slices of functional images covering the whole brain acquired using a gradient-echo echo-planar imaging (EPI) pulse sequence, slice thickness = 3 plus interslice gap = 0.5 mm, 64×64×37 matrix with a spatial resolution of 3.5×3.5×3.5 mm, TR = 3000 ms, TE = 30 ms, FOV = 205 x 205 mm^2, flip angle = 90°, in plane resolution = 3.2 x 3.2 mm^2.

2.2.6 Data processing and statistical analysis

Data analysis was performed with SPM12 (Wellcome Department of Imaging Neuroscience, University College, London, UK; http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2016 (The Mathworks, Inc.). Structural images were manually centered and reoriented with functional images to the anterior-posterior commissure axis. The first four EPI volumes of each functional run were discarded to allow the magnetization to reach a steady state. For each subject, all volumes were slice timing corrected, spatially realigned to the first volume of the first functional run and un-warped to correct for between-scan motion. Motion parameters were used as predictors of no interest in the model to account for translation and rotation along the 3 possible dimensions as determined during the realignment procedure. Individual dataset was excluded if excessive head motion was observed (translation > 3 mm or rotation > 3°). T_1-weighted image was segmented into gray, white and cerebrospinal fluid and spatially normalized to the Montreal Neurological Institute (MNI) space. Spatial transformation derived from this segmentation was then applied to the realigned EPIs for normalization and re-sampled in 2×2×2 mm^3 voxels using trilinear interpolation in space. All functional volumes were then spatially smoothed with a 6-mm full-width half-maximum isotropic Gaussian kernel.

Data were analysed using a random-effects model (Friston et al. 1999) implemented in a two-level procedure. In the first-level, single subject fMRI responses were modelled using a General Linear Model (GLM), for which a design-matrix included the onsets and durations of each event for each condition. The model combined the two action observation runs, modelling five predictors corresponding to experimental and control action observation conditions (*AO Novice, AO Intermediate, AO Expert, AO Ctrl and Response*) plus six predictors obtained from the motion correction in the realignment process to account for voxel intensity variations caused by head-movement and one constant regressor per run. All predictors, except for *Response*, included the five consecutive videos of each trial, which were modelled as one single epoch lasting 15-s. Catch trials were modelled as consecutive blocks, lasting 15-s each, comprising the effective response time (3-s) and a signal-denoising period (12-s) to separate the motor component from subsequent processing.

In the second level group-analysis, corresponding *t*-contrast images (*AO Novice* vs. *rest*, *AO Intermediate* vs. *rest*, *AO Expert* vs. *rest*, *AO Ctrl* vs. *rest*) of the first-level models were entered in a flexible ANOVA with sphericity-correction for repeated measures (Friston et al. 2002). Within this model, we also assessed the activations resulting from the direct contrasts between conditions (*AO Novice* vs. *AO Ctrl*, *AO Intermediate* vs. *AO Ctrl*, *AO Expert* vs. *AO Ctrl*, and all reverse contrasts) with a statistical threshold of $P < 0.001$, with family wise error (FWE) correction at cluster level. Finally, in order to assess brain region modulated by expertise effects we computed direct contrasts between conditions (*AO Novice* vs. *AO Intermediate, AO Novice* vs. *AO Expert* and all reverse contrasts) with a statistical threshold of $P_{FWE} < 0.001$ corrected at cluster level).

Data corresponding to the action execution Localizer were analysed using a GLM with two predictors (*Act Pre* vs. *rest*, *Act Exe* vs. *rest*) convolved with the HRF. This latter contrast was used for the localization of regions of interest (ROIs, see next section) belonging to the parieto-premotor MNS, involved also in both action execution and observation. Results of the Localizer were thresholded at $P_{FWE} < 0.05$, corrected for multiple comparisons at the voxel level for GLM univariate analysis or $P_{FWE} < 0.001$ at the cluster level for the localization of individual activation foci within the parieto-premotor MNS. Local maxima of activation are presented in the stereotaxic space of the MNI coordinate system.

ROI-based analysis

The regions of interest (ROIs) were defined using the action execution Localizer at a multi-subject level in order to restrict the analysis to the main node of the MNS. ROIs were selected according to the following criteria: 1) activated voxels overlapped for the four experimental conditions (*AO Novice, AO Intermediate, AO Expert and AO Ctrl*) masked with the contrast image derived from the action execution Localizer (*Act Exe* vs. *rest*); 2) the masked overlapping activations had to survive a threshold of $P_{FWE} < 0.001$, corrected at cluster level; 3) voxels were in anatomical regions belonging to the MNS, identified in the literature as being involved in hand-object manipulative actions as well as action observation, namely, superior parietal lobule (SPL), intraparietal sulcus (IPS), dorsal premotor cortex (PMd) and ventral premotor cortex (PMv). Anatomical descriptions were, in the majority of the cases, performed based on the probabilistic cytoarchitectonic maps of the brain mapping group in Juelich, Germany (Geyer et al. 1996, 1999, 2000; Grefkes et al. 2001; Geyer 2003; Eickhoff et al. 2005; Caspers et al. 2006) as implemented in the SPM12 anatomy toolbox; Eickhoff et al. 2005). Four ROIs were defined centring a sphere (radius 4-mm) in the left SPL (ROI 1: $x = -28$, $y = -56$, $z = 58$, SD ± 3 mm), left PMd (ROI 2: $x = -26$, $y = -8$, $z = 54$, SD ± 3 mm), left IPS (ROI 3: $x = -36$ $y = -40$ $z = 48$, SD ± 3 mm) and left PMv (ROI 4: $x = -54$, $y = 8$, $z = 30$, SD ± 3 mm) respectively. Then, for each subject the signal change was extracted using the SPM Rex Toolbox (http://web.mit.edu/swg/rex), that permitted to extract the signal change values in the ROIs for each participant on the basis of contrast images (second-level analysis: *AO Novice* vs. *rest*, *AO Intermediate* vs. *rest*, *AO Expert* vs. *rest*, *AO Ctrl* vs. *rest*) based on the previous flexible ANOVA model.

Regression Analysis

To investigate the relationship between functional activations changes within the AON/MNS areas and the level of own finger/manual dexterity assessed with the PPT, the averaged individual normalized PPT scores were correlated (Pearson) with the BOLD signal change extracted in ROIs belonging to the parieto-premotor AON/MNS, localized using both the action observation and action execution tasks. In particular, we performed a conjunction analysis to highlights voxels activated either during the action observation of expert model (*AO Expert* vs. *rest*) and action execution (*Act Exe* vs. *rest*) (Figure 15). Using the conjunction analysis map, four ROIs were defined centring the sphere (radius 4-mm) at the local maxima in SPL, PMd, IPS and PMv regions. Then, as mentioned above, the BOLD signal change associated with the observation of expert model was extracted in the four ROIs for each subject. It is important to note that the individual behavioural score entered in the regression analysis was related exclusively to the execution of the PPT task performed using the right dominant hand, spatially compatible with that observed by participants from a first-person perspective during the fMRI action observation task.

Psycho-physiological Interaction Analysis (PPI)

We performed a psycho-physiological interaction analysis (PPI) (Friston et al. 1997; Friston 2011) to investigate a possible modulation of the observation condition (*AO Novice* vs. *AO Expert*) on the functional connectivity pattern between brain areas involved in the processing of complex hand-object manipulation. The purpose of a PPI analysis is to determine which voxels in the whole-brain increase their relationship with a seed region of interest in a given context, such as during a particular behavioral task. In other words, PPI aims to identify regions whose activity depends on an interaction between psychological and physiological factors (the BOLD time course of a region of interest). A task-specific increase in the relationship between brain regions (a PPI effect) is suggestive of a task specific increase in the exchange of information (O’Reilly et al. 2012). In this study, PPI analysis allowed us to highlight brain regions whose activity depends on an interaction between action observation of the novice model and action observation of the expert (Psychological task) and the BOLD signal change of the superior parietal lobule during the contrast [*AO Novice* (*t*) versus *AO Expert* (*t*)] (Physiological response). The PPI procedure was performed in 3 different steps: 1) extraction of the BOLD signal in a seed ROI; 2) PPI analysis; 3) PPI GLM analysis.

Step 1. For each participant, the seed region (local maxima) was localized in the left SPL (x = −28, y = -56, z = 58; SD ± 3 mm). This yielded 18/18 participants with valid seed regions. In particular, seed area was identified at single subject level on the basis of the first-level GLM results obtained using the action execution Localizer. Then, using the SPM12 *eigenvariate* function, we extracted for each subject the time course of activity in a volume of interest (VOI) centering a 4-mm radius sphere around the local maxima identified before.

Step 2. The PPI analysis employed 3 regressors as follows: one regressor represented the deconvolved activation time course (Gitelman et al. 2003) in the SPL seed (Y vector, physiological variable), one regressor represented the contrast of interest [*AO Novice* (*t*) vs. *AO Expert* (*t*)] (P vector, psychological variable), and one regressor represented the interaction of the previous two vectors (PPI, the interaction term). PPI analysis assessed the connectivity between seed and whole-brain by multiplying the deconvolved BOLD signal with psychological vector.

Step 3. After deconvolution of HRF, for each participant, the 3 regressors (PPI term, Y vector, and P vector) were entered into a first-level GLM to determine individual brain regions showing psycho-physiological interaction with the SPL seed regions. As covariates of no interest, models also included the six motion parameters. To investigate PPI effects at multi-subject level, we entered contrast images of the PPI effects for each participant into a random-effects analysis using a flexible factorial repeated-measures ANOVA (within-subject factor: level of expertise; blocking factor: subject).

2.3 Results

Brain representation of complex hand action observation

The observation of complex hand-object manipulation versus baseline (*AO Novice*, *AO Intermediate*, *AO Expert*) produced common activations in areas belonging to the parieto-premotor MNS (Figure 10). Figure 10 shows averaged multi-subject activations displayed on a cortical high-resolution MNI template available on Conn Toolbox for SPM12. The maps were generated using the contrast images *AO Novice* versus *rest*, *AO Intermediate* versus *rest* and *AO Expert* versus *rest* (see Methods).

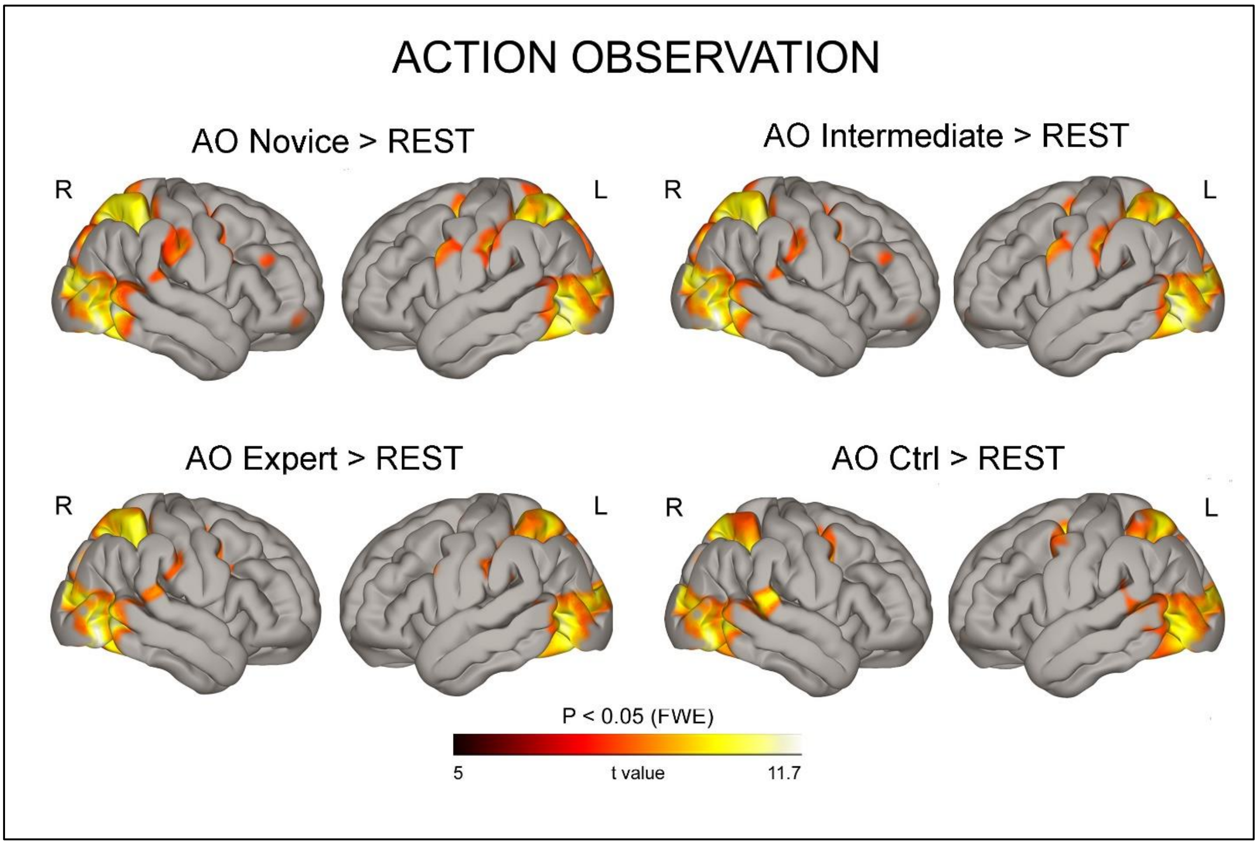


Figure 10: Brain activations resulting from the experimental conditions and relative control during the action observation sessions: action observation Novice vs. Baseline (AO Novice vs. Rest), action observation intermediate vs. Baseline (AO Intermediate vs. Rest); action observation Expert vs. Baseline (AO Expert vs. Rest), Ctrl Observation vs. Baseline (AO Ctrl vs. Rest). All activations are rendered into a standard Montreal Neurological Institute brain template (P_{FWE} <0.05 voxel level). L, left hemisphere; R, right hemisphere.

All the activations revealed in the group analysis has also been analysed using a statistical threshold of $P_{FWE} < 0.05$, corrected at voxel level ($t > 5$). The activation pattern was largely symmetrical, although some clusters were present only in one hemisphere. Common activations included clusters in occipito-temporal, parietal and premotor cortices. The occipito-temporal activation reached maximum near the posterior part of the inferior temporal sulcus (ITS) and the posterior middle temporal gyrus (pMTG). This activation showed two branches, one extending dorsally to the posterior end of superior temporal sulcus (pSTS), and a ventral branch extending into the occipito-temporal sulcus (OTS). The parietal activation included the inferior and superior parietal lobules (IPL/SPL), plus the ventral, dorsal and anterior intraparietal sulcus (vIPS, dIPS, aIPS). In the frontal lobe, common activations were found in the dorsal and ventral premotor cortices (PMd / PMv), plus the inferior frontal gyrus (IFG, BA44) and the dorsolateral prefrontal cortex (DPFC) in the right hemisphere. Subcortical activation included also thalamus bilaterally, putamen caudate nucleus.

The activation clusters resulting from the observation of control movements (*AO Ctrl* versus *rest*; Figure 10), consisting in simple fingers movement without objects, revealed a less extended symmetrical pattern belonging to the parieto-premotor MNS. In particular, activated clusters included bilateral occipito-temporal cortex (STS, MTG, IFG), posterior parietal cortex (SPL, IPS) and, in the frontal lobe, an area at the border between PMV and PMd cortex.

Observation of action performed with different expertise versus control

Compared to control observation condition, consisting in simple fingers movements, observation of complex hand object manipulation performed by a novice actor without particular manipulation skills, activated strongly the AON/MNS including the ITG, the IPL, supramarginal gyrus (SMG), IPS and the SPL, the PMd cortex, and the IFG plus the VPFC in the right hemisphere (Figure 11; Supp. Table 3; $P_{FWE} < 0.001$, corrected at cluster level). Other regions showing consistent activation were the lobule VI of the cerebellum, bilaterally, and the cingulate cortex. MNI coordinates for all the experimental conditions vs. control are reported in Supp. Table 4.

The comparison between the observation of complex manipulative actions performed by an actor with an intermediate hand manipulation skill versus control observation showed clusters of activation localized in regions common to the observation of novice, including the IPL / SMG and the IPS that belong to the parietal region, and the PMd cortex in the frontal lobe, plus the

IFG exclusively in the right hemisphere. Other regions activated were the MTG / ITG, the cingulate cortex and the cerebellum.

The comparison between the observation of object manipulative actions performed by the expert actor with high hand skills versus control observation revealed a less extended activation within the MNS regions, with respect to the other experimental conditions. Regions consistently activated were the PMd cortex bilaterally and the IFG in both hemispheres, revealing a stronger response of this region during the observation of skilled actions. In the parietal region, activation clusters were found in the IPL / SMG and the IPS bilaterally. However, the SPL region was more strongly activated in the right hemisphere, compared to left one.

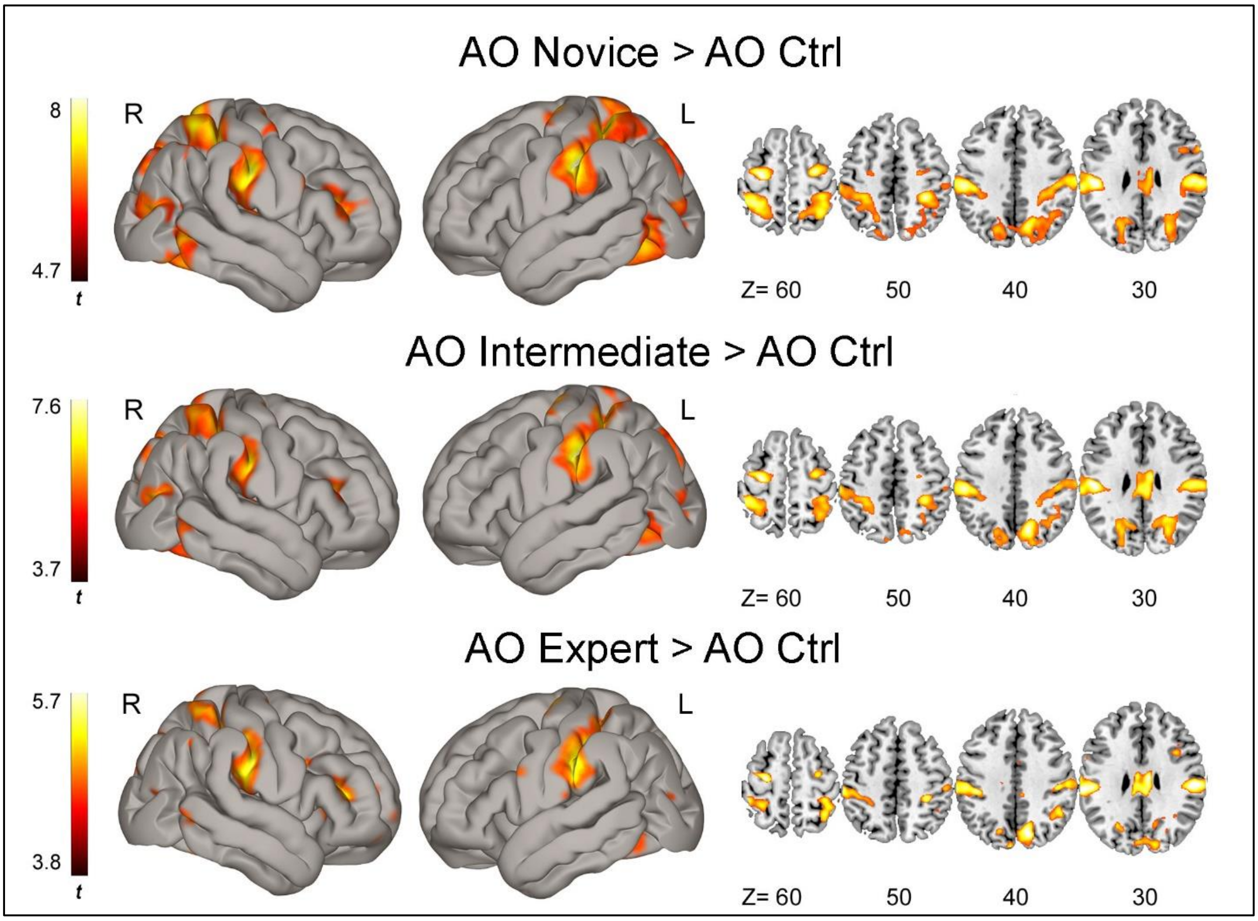


Figure 11: Brain activations resulting from direct contrasts between experimental conditions during the action observation sessions: action observation Novice vs. observation Control (AO Novice vs. AO Ctrl), action observation Intermediate vs. observation Control (AO Intermediate vs. AO Ctrl); action observation Expert vs. observation Control (AO Expert vs. AO Ctrl). All activations are rendered into a standard Montreal Neurological Institute brain template (P_{FWE} <0.001 cluster level) and in axial slices of reference. L, left hemisphere; R, right hemisphere.

Finally, we directly contrasted brain activation induced by observation of novice actor versus observation of expert model (Figure 12 A; Supp. Table 4). The comparison between the two conditions revealed a parieto-frontal dorsal network of areas mostly lateralized to the left hemisphere including the SPL and the IPS within the parietal region, the PMd cortex and other clusters of activation in the ITG bilaterally. The inverse contrast and the others possible comparison did not show any other difference between conditions.

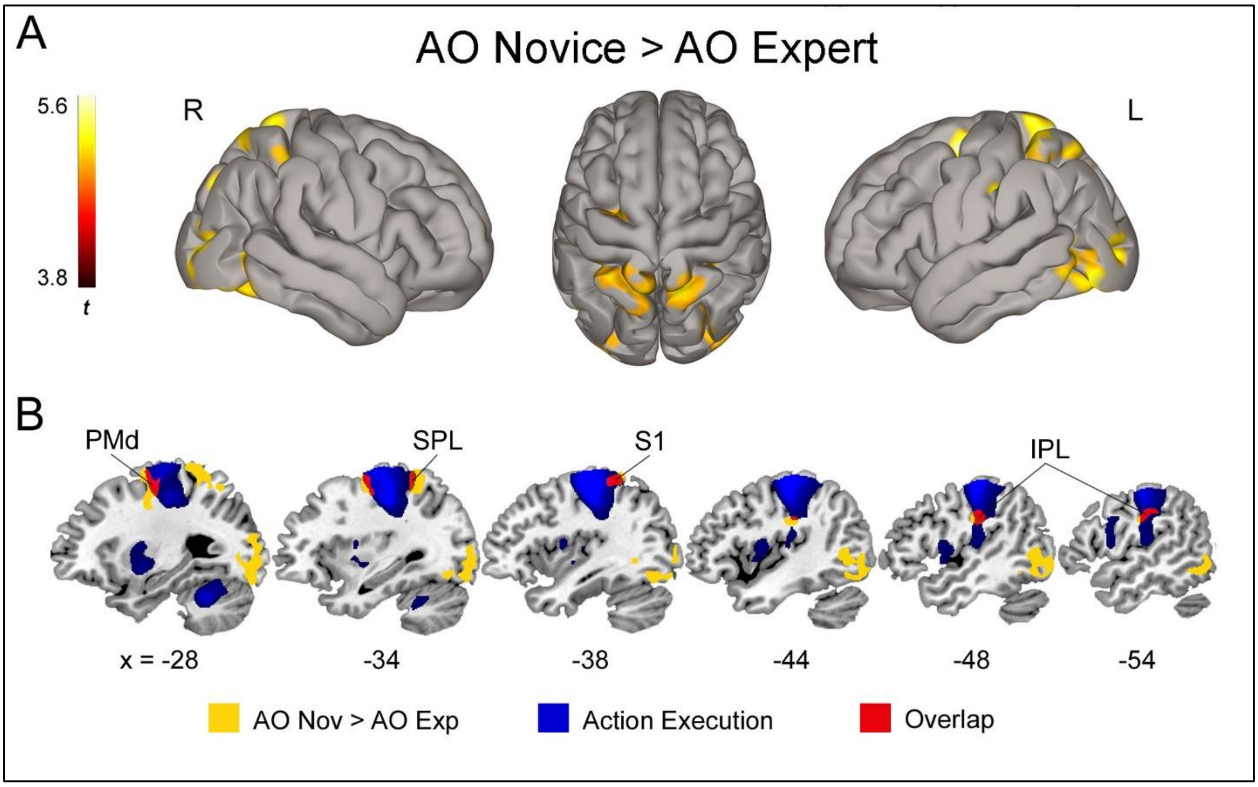


Figure 12: A) Brain areas activated by observation of actions performed by novice model compared to the expert one (AO Novice vs. AO Expert). B) Overlap of activations resulting from the direct contrast AO Novice vs. AO Expert (yellow color) AND the activation clusters during action execution Localizer (blue color). All activations are rendered into a standard Montreal Neurological Institute brain template (P_{FWE} <0.001 cluster level). Abbreviations: IPL, inferior parietal lobule; L, left hemisphere; PMd, dorsal premotor cortex; R, right hemisphere; S1, primary somatosensory area; SPL, superior parietal lobule;

Action execution Localizer

In order to restrict our analysis to the main areas belonging to the MNS, we identified regions activated during motor execution. It is known from previous studies (see Binkofski et al. 1999) that manipulating an object involves motor as well sensory components, and it is therefore expected to activate several brain areas subserving this function.

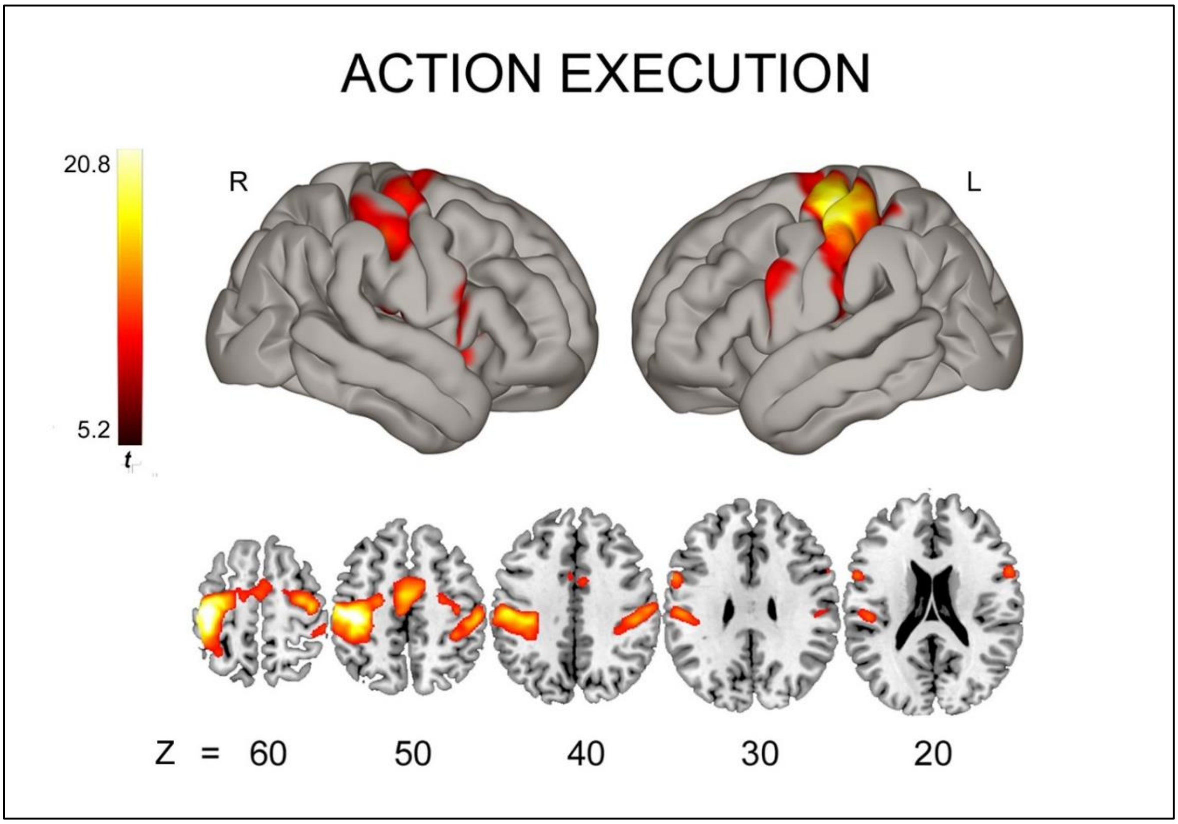


Figure 13: Brain activations resulting from the Localizer during action execution (Act Exe vs. rest). The activations are rendered into a standard Montreal Neurological Institute brain template (P_{FWE} <0.05 voxel level). Axial slices correspond to five representative sections. L, left hemisphere; R, right hemisphere.

Indeed, brain activation in this study were observed both at a multi-subject level ($P_{FWE} < 0.05$, corrected at voxel level) and in single-subject maps ($P_{FWE} < 0.001$, corrected at cluster level). These brain areas included: primary motor cortex (M1), primary somatosensory cortex (S1), PMd and PMv cortices and the IFG, the supplementary motor area (SMA), cingulate cortex, IPL / SPL and the IPS (Figure 13 for a multi-subject map). Most activations were lateralized or stronger in the left hemisphere compared to the right one, as expected for normal right-handed participants moving their right hand. Primary motor cortex activation was strongly lateralized to the left hemisphere, as well to SI, extended to SII region. Interestingly, activations of PMv cortex and IFG were greater compared to the PMd cortex, consistent with the fact that the executed actions involved hand grasping/manipulation. We used multi-subject maps to localize MNS areas involved in execution of complex object manipulation in order to extract BOLD timecourse related to action observation conditions. At a single subject level, we also localized the SPL activation at local maxima as seed region for the subsequent functional connectivity analysis (see PPI analysis). Figure 12 B shows (in red color) an overlap between action execution map resulting from the MNS Localizer and the contrast image derived from the direct comparison between observation of novice versus expert. Overlapping regions were found in SPL, SMG / IPL, SI and PMd.

ROI Analysis

The most important comparison was that performed in order to evaluate a possible differential response of the MNS during action observation in relation to the expertise of the model observed in the videos. To do this, a ROI analysis has been performed in four parieto-frontal areas activated by the action execution Localizer at a multi-subject level and considered involved both in action observation and execution (left SPL, left PMd, left IPS and left PMv; Fig. 14 A). The identified these ROIs are in agreement with previous metanalysis on action observation in humans (see Caspers et al. 2010; Molemberghs et al. 2012) and previous studies showing overlap in activation during action observation and execution (Buccino et al. 2001; Gazzola and Keysers, 2009).

Furthermore, in accord with the notion of the mirror mechanism, the four identified regions have separate functional properties. In fact, observed proximal reaching movements are typically associated with the activation of dorsal parieto-frontal circuit linking the convexity of the SPL to the PMd cortex. On the contrary, several neuroimaging studies on action observation confirmed that the processing of object-centred visually guided hand actions relies on a ventral parieto-frontal circuit including the anterior IPS and the IPL, and the PMv cortex plus the posterior part of the IFG. According to this distinction we included in the analysis the four more representative regions belonging to the dorsal and ventral parieto-frontal circuits. The averaged hemodynamic response (histograms of Figure 14 B) and the relative time course (plots of Figure 14 B) relative to the four conditions (*AO Novice, AO Intermediate, AO Expert, and AO Ctrl*) have been analysed in the four ROIs.

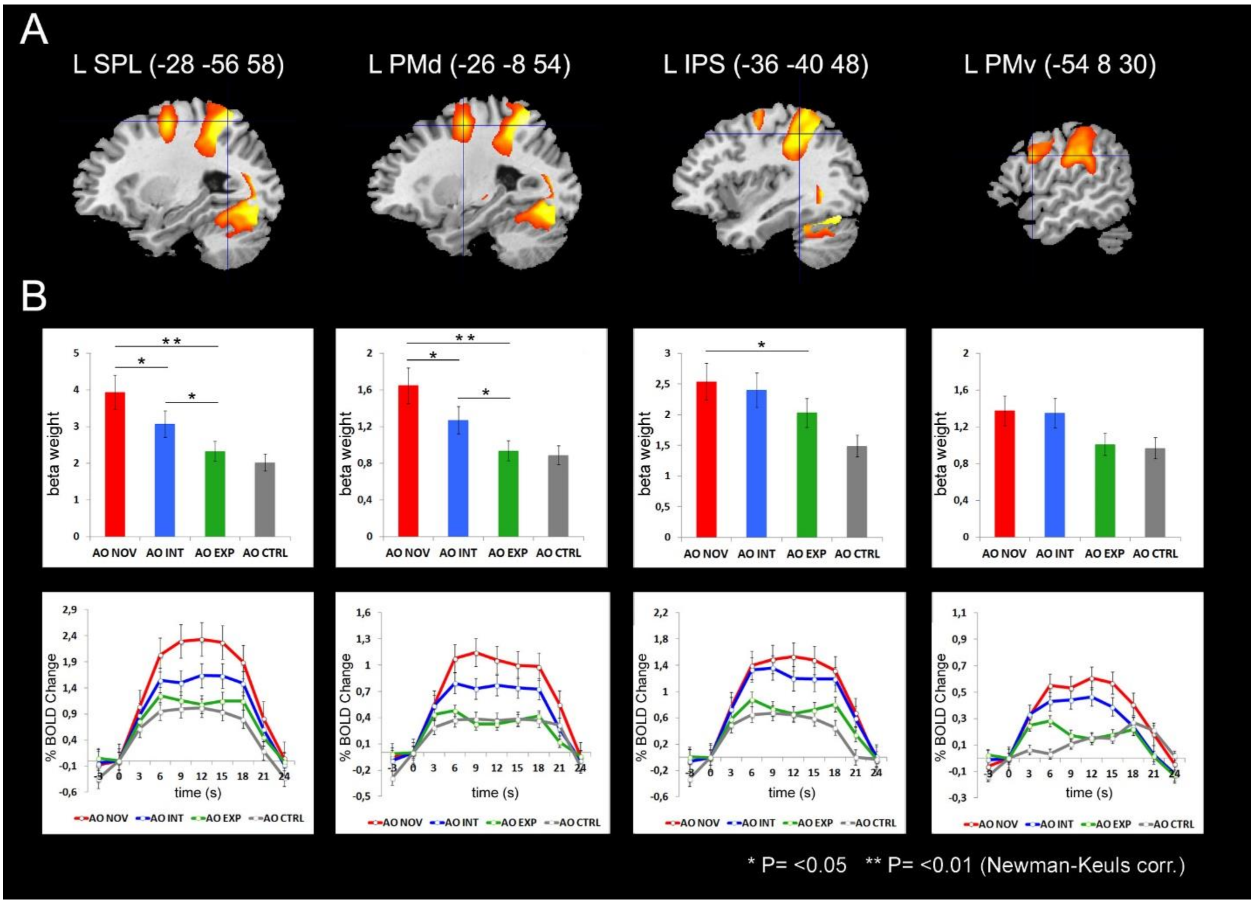


Figure 14: Brain activations during action observation. A) Parasaggittal sections showing the activations resulting from the conjunction between AO Novice AND AO Intrmediate AND AO Expert, masked with an inclusive contrast image corresponding to action execution activations (P < 0.05). Crosshair indicate the centre of ROIs identified belonging the MNS. B) Histograms show the averaged magnitude of activation (parameter estimate) in each ROI. Line graphs indicate time-course response across peri-stimulus time in left SPL, PMd, IPS and PMv ROIs for all the experimental and control conditions. Bars indicate SEM. Asterisks indicate significant differences set at *P* < 0.05 (*) and *P* < 0.01 (**).

Results were examined using analysis of variance and Newman-Keuls post-hoc comparisons. The ROI analysis of the parietal activations showed that left SPL (Figure 14) was more active following the observation of hand object manipulative actions performed by the novice actor, compared to that executed with an intermediate level of expertise (P < 0.05) and high expertise (P < 0.01). In addition, a significant difference was found in SPL region between activation during observation of intermediate compared to expert (P < 0.05) consisting of a graduate incremental response for different levels of motor resonance. These findings emerge from the analyses of both the average signal change and the time course of the signal during the presentation of the stimuli. Similar results were obtained when exploring the hemodynamic response in PMd cortex during the four experimental conditions. A comparison between the BOLD signal changes measured in the action observation conditions showed very similar incremental response for different level of expertise, with stronger response during the observation of novice model compared to intermediate ($P < 0.05$) and expert ($P < 0.01$). Furthermore, similarly to SPL, even in PMd cortex BOLD change was greater for the observation of intermediate model compared to the expert ($P < 0.05$). A significant effect was found also in IPS ROI, showing a stronger BOLD response for actions performed by novice compared to expert ($P < 0.05$). However, in the IPS region no difference was found between activations between the novice vs. intermediate or expert vs. intermediate conditions. Finally, in the PMv cortex ROI there was no difference between BOLD activations in the four experimental conditions. Possibly, these results indicate that regions considered in the analyses are involved in different aspects of action processing.

Regression Analysis

Percent signal change in three of four identified ROIs belonging to the AON/MNS positively correlated with the PPT scores obtained by participants using the right dominant hand (Figure 15). During the observation of the expert model performing complex manipulative actions, subjects demonstrated a significant positive correlation between BOLD signal change in the left SPL ($r = 0.52$, $P < 0.01$), left IPS ($r = 0.47$, $P < 0.05$) and left PMd ($r = 0.56$, $P < 0.01$). On the contrary, there were no significant correlations between PPT individual scores and PMv activity ($r = 0.07$, $P > 1$, n. s.).

PPI analysis

To determine which brain areas have a specific role in motor resonance induced by the observation of actions performed with high level of manual expertise, we adopted a functional connectivity approach by using a PPI analysis, requiring a psycho-physiological interaction between a seed region and the whole-brain, voxel-by-voxel. In particular, we conducted a PPI analysis (Friston et al. 1997) to test the hypothesis that functional connectivity between SPL and other areas in the parieto-premotor AON/MNS would be greater when a participant observe actions already embodied in the personal own motor repertoire, like daily life actions that the individual can perform, compared to complex skilled actions.

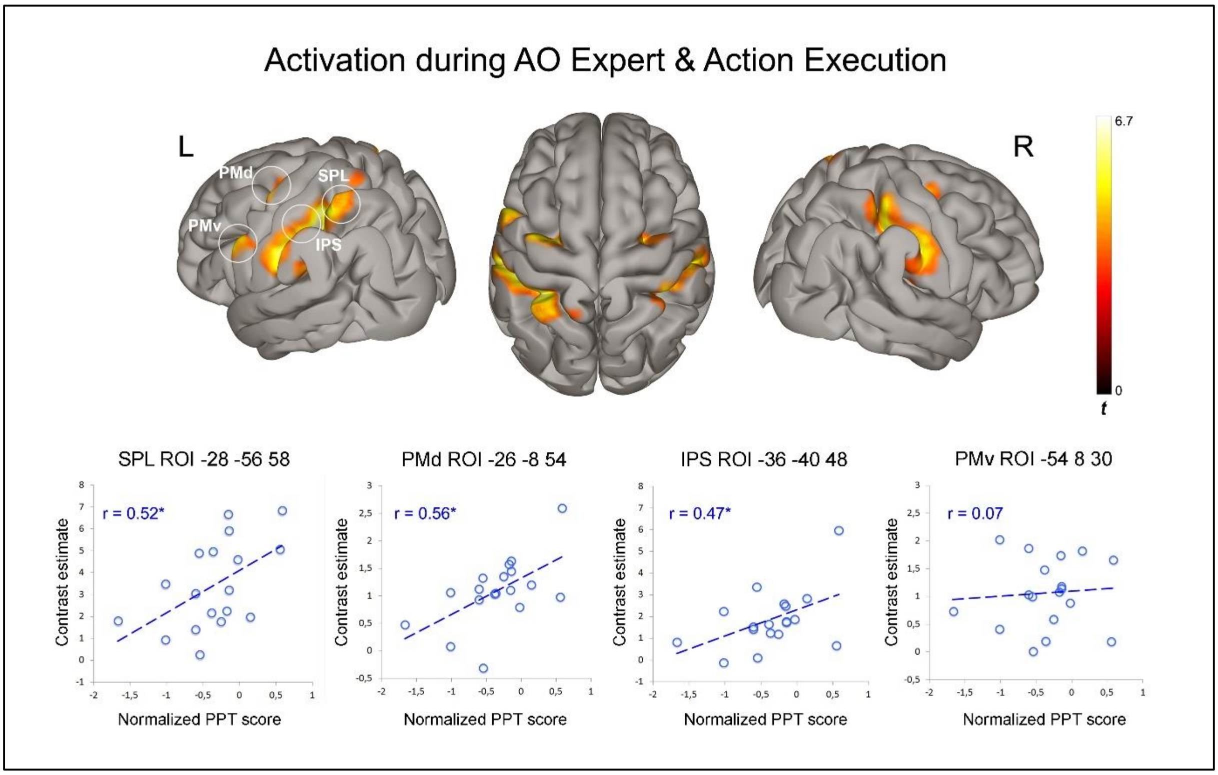


Figure 15: Regression Analysis. Brain activations resulting from the conjunction analysis between AO Expert AND Act Exe Localizer, rendered into a MNI standard template. Scatterplots below show the correlation between BOLD signal change during the AO Expert condition and the normalized PPT individual score obtained by participants using the right hand (hand ability score). On the top of each plot are reported MNI coordinates for each of the four ROI. L = Left, R = Right.

As seed we used the left SPL that showed a strong activation both during the three action observation conditions and the action execution Localizer. We tested the hypothesis that this area would be functionally coupled with bilateral parietal and frontal MNS areas found in this study to be selective for the observation of actions within the own motor repertoire, like the PMd cortex and the IPS.

As displayed in Figure 16, left SPL demonstrated significantly increased functional coupling with clusters in the left hemisphere as left PMd cortex (peak: $x = -30$, $y = -10$, $z = 58$; $t = 5.88$; cluster-level $P_{FDR} < 0.03$), left IPS (peak: $x = -36$, $y = -48$, $z = 48$; $t = 6.12$; cluster-level $P_{FDR} < 0.006$), left MTG (peak: $x = -56$, $y = -60$, $z = -2$; $t = 6.05$; cluster-level $P_{FDR} < 0.02$). Moreover, left SPL was functionally connected with areas in the right hemisphere like right SPL (peak: $x = 38$, $y = -52$, $z = 58$; $t = 5.60$; cluster-level $P_{FDR} < 0.0001$), right IPS (peak: $x = 32$, $y =$ -

46, $z = 48$; $t = 6.48$; cluster-level $P_{\mathrm{FDR}} < 0.001$) and right MTG (peak: $x = 52$, $y = -58$, $z = -2$; $t = 4.92$; cluster-level $P_{\mathrm{FDR}} < 0.001$). This demonstrate that the observation of actions performed by the novice model, already present in the motor repertoire of participants, not only activates the MNS but also modulates the connectivity between left SPL and the other parieto-premotor areas.

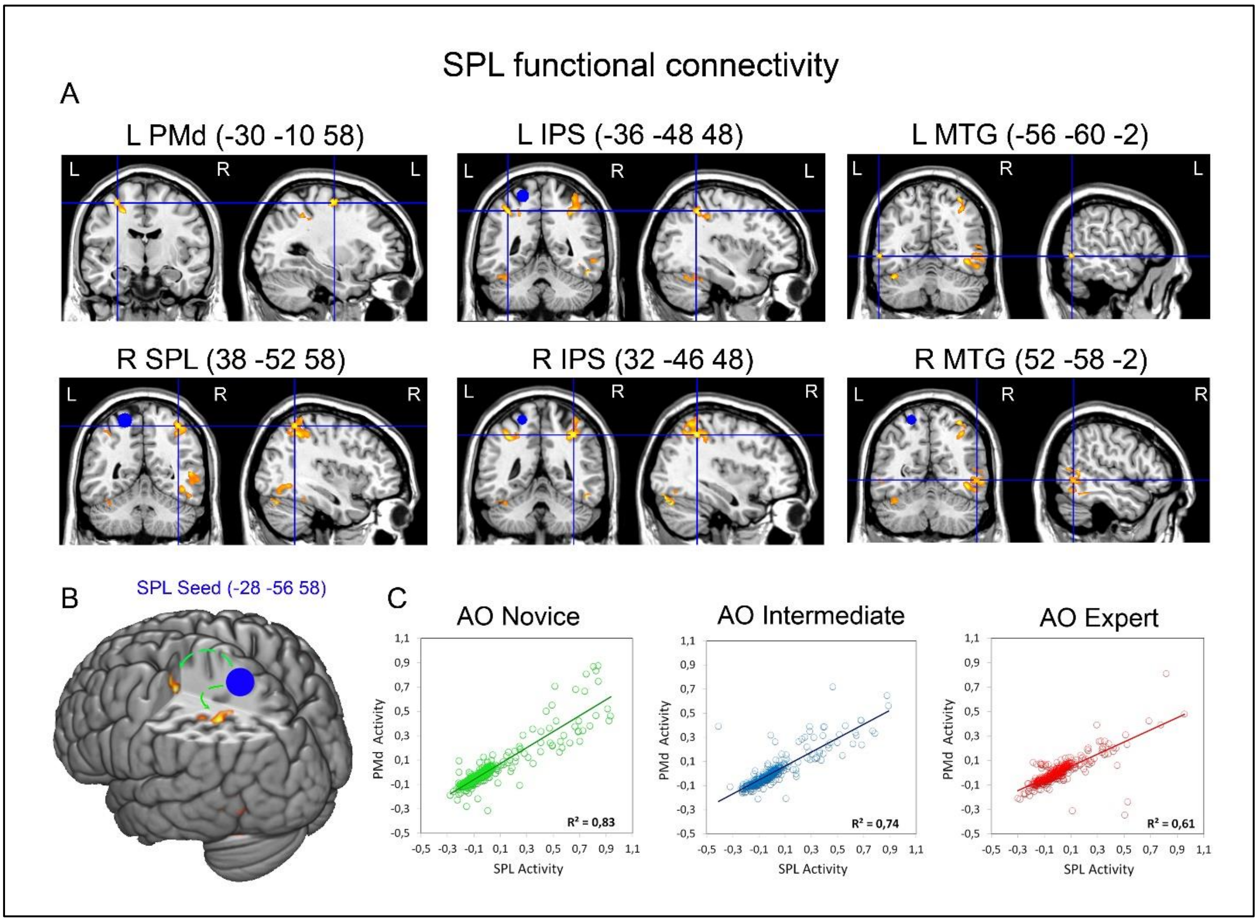


Figure 16: PPI results. A) Several brain regions increase their functional connectivity with SPL seed region during the observation of novice model compared to the observation of expert. Activation are shown at $P_{\mathrm{FWE}} < 0.001$, corrected at cluster level. B) Seed region in SPL is shown as blue color sphere and activations of functionally connected areas are rendered into a standard 3D MNI template. C) Plots of correlations between brain activity (BOLD signal change) in SPL region and PMd cortex during the three observation conditions.

However, it is important to note that left SPL appeared to be strongly connected with regions belonging to the MNS except for the inferior frontal areas considered part of the ventral parieto-frontal network for visuo-motor transformation associated particularly with grasping actions. These findings are discussed with more details in the next session.

2.4 Discussion

The aim of this study was to determine whether the observation of actions performed with different levels of expertise is able to modulate the activations of areas belonging to the parieto-premotor MSN/AON, in a group of healthy individuals without specific motor skills. Only the results surviving voxel-level FWE correction at $P < 0.05$, or cluster level FWE correction at $P < 0.001$ are discussed. Our results showed that the SPL region, IPS and PMd cortex were strongly activated in healthy participants during the observation of complex object manipulation performed by a naïve subject without particular hand skills. Moreover, the BOLD activation within the left SPL, left IPS and left PMd during observation of actions performed by the expert was positively correlated with individual hand skill dexterity, assessed with behavioral standardized test. Furthermore, functional connectivity during the observation of the naïve model compared with expert, revealed activation within the dorsal AON, including the SPL and other areas anatomically and functionally coupled to it, like the PMd cortex. This suggests a possible distinction between ventral and dorsal brain circuit involving in the processing of different aspects of action perception, such as kinematic features and final goal of the observed actions.

Brain representation of actions performed with different levels of expertise

In this study, we demonstrated that observing complex manipulative hand actions performed with a level of expertise similar to that of the observer is able to enhance activation of the MNS in healthy people. These findings could, in principle, be attributed to the basic features of employed stimuli or to unspecific factors. One of these features could be the effective duration of the observed hand-object manipulation, which was dissimilar between the three conditions. However, the absence of differences in motion energy quantification between conditions indicates that this explanation can be excluded, because there was no correspondence between duration of movement and motion energy. Another possible confounding factor could be the visual familiarity with the observed model. However, all the models were unfamiliar for the naïve participants, because both the expert and the novice model performed unfamiliar complex manipulative actions.

The experience with a particular motor skill seems to play a key role in the modulation of the parieto-premotor MNS during the observation of actions requiring that motor skill. Previous neuroimaging studies used two main approaches to investigate this issue. The first approach is to study population of experts (e.g. elite sportsmen, dancers, piano players etc.) who have particular motor skills (Calvo-Merino et al. 2005, 2006; Cross et al. 2006, 2009; Aglioti et al. 2008; Abreu et al. 2012). Usually, studies on the role of the MNS in hyper-specialized populations found a stronger activation for the expert in that field compared to novices or expert in other fields (Calvo-Merino et al. 2005; Abreu et al. 2012). Another approach consists in the recruitment of homogeneous populations of naïve subjects, without particular motor skills, observing actions performed by agents (models) with different expertise (Buccino et al. 2004; Gazzola et al. 2007; Cross et al. 2012). The present study employed the second approach, exploring brain activity in a group of healthy volunteers, assessed behaviorally for hand motor skills, resulting in a mean range of manual dexterity, taking into account demographic characteristics like age and educational degree. Our neuroimaging findings showed that during the observation of complex manipulative actions performed with different degree of expertise, the MNS of the naïve observers tends to be strongly activated if the actions are close to the individual own motor repertoire (that is the novice model). It is important to note that we used a biological effector (a right hand) performing manipulative actions with increasing complexity, with regard to previous study contrasting actions performed by agents with different appearance, i.e. different species (Buccino et al. 2004), robotic versus human agents (Tai et al. 2004; Gazzola et al. 2007a; Cross et al. 2012) and biological possible versus impossible movements (Costantini et al. 2005). In a previous fMRI investigation, Buccino and coworkers studied brain activity in normal volunteers observing video-clips showing mouth motor acts made by humans, monkeys, and dogs. Their data demonstrated that the left IPL and IFG responded to actions made by human and nonhuman performers, as long as the action was part of the human motor repertoire (e.g., biting). In contrast, there was no activation (barking) or almost no activation (lips-making) when the action belonged to another species (Buccino et al. 2004). This is in line with our findings, even if we used as stimuli only biological real hands, corresponding to three actors with different level of manual dexterity: novice, intermediate and expert. This suggests that motor resonance induced by action observation is not an all-or-nothing process but involves different levels of mirroring with the observed model. Moreover, observing models with different skills may induce differential responses within the MNS in spite of the similarity in the appearance among models. Another important aspect to consider concerns the actions chosen in this study. We used complex manipulative actions consisting in moving a small object between fingers, which require a high level of control and high speed of finger movements. This is also an innovative approach compared to previous neuroimaging studies on expertise using whole body actions like dancing and sports. Our results highlight the role of the MNS in motor resonance specifically induced by observation of finger/hand dexterity, and demonstrate the incremental modulation of the mirror mechanism when there was a correspondence between observer's motor repertoire and observed model.

Representation of expertise in the dorsal parieto-frontal network

Our findings showed that distinct neural circuits belonging to the MNS/AON could process actions performed with different levels of expertise. In particular, by contrasting brain activation induced by the observation of manipulative actions performed by the novice versus expert model, we found a dorsal network of areas constituted by the SPL region, the PMd cortex and the middle IPS, selectively modulated by actor's motor expertise. This finding emerges also from the psycho-physiological interaction analysis performed to highlight which voxels in the whole-brain are functionally coupled with the SPL seed region, during the processing of actions executed by the novice actor versus the expert one. In addition, this analysis confirmed the univariate results, showing the functional connectivity between the SPL and other brain regions in the MNS/AON, in particular the PMd cortex. These results are partly different from previous neuroimaging and neurophysiological studies in humans suggesting that the parieto-premotor MNS encode uniquely the goal of the observed actions. For instance, Gazzola and coworkers (2007a) reported that frontal and parietal areas are activated by the observation of actions performed by a real human arm as well as by a robotic arm, although human and robotic effectors differ in terms of both appearance and kinematic features. Furthermore, a study on aplasic subjects provides evidence in favour of goal coding within the MNS (Gazzola et al. 2007b). The authors investigated if the goal of a hand movement could be recognized in the absence of any experience of hand movements. To address this issue, two individuals born without arms and hands were studied. While being scanned, they were asked to watch video-clips showing hand actions and their brain activations were compared with those of control volunteers. All participants also made actions with different effectors (feet, mouth and, for normal volunteers, hands). The results showed that the MNS of aplasic individuals was activated by the observation of hand motor acts. This demonstrates that the brains of aplasics can mirror motor acts that they have never executed. The goal is recognized through the recruitment of areas involved in the execution of motor acts having the same goal but using different effectors, such as mouth and foot.

On the other hand, our findings are in line with other studies demonstrating that, unlike in monkeys, the human MNS/AON is activated not only by goal-directed actions but also by intransitive actions, i.e. fingers or arm movements, being sensible to kinematic parameters (Fadiga et al. 1995; Wheaton et al. 2004; Urgesi et al. 2006; Alaerts et al. 2009; Casile et al. 2010). For instance, Casile and coworkers (2010) investigated the neural substrates involved in the processing of motor invariants during action observation. They compared the BOLD responses during the observation of intransitive movements complying with or violating the two-thirds power law of the movement and their results showed higher activations within the PMd cortex, the middle superior frontal gyri and medial frontal cortex. This suggests that a network of regions within the dorsal MNS/AON is involved in the processing of normal kinematic features of human motion or possibly features of actions already embodied in the personal motor repertoire. Given these properties of the human MNS, one can argued that sensorimotor activation in humans during action observation could be explained by different mechanisms for action perception (Hamilton and Grafton 2006, 2008). In line with this idea, actions can be described at different levels (Grafton and Hamilton 2007): 1) the kinematic level, including the processing of reach trajectory, velocity, grip configuration and means, that describe dynamic interactions based on object weight and specific manipulation of an object; 2) the goal-object level, that is the understanding of “what” the other is doing (e.g., grasping an object); 3) the outcome level, that is defined by the physical consequences of an action (i.e. changing the position of objects in the world). On this assumption, Hamilton and Grafton (2006) used repetition-suppression technique (RS) to distinguish different levels of action representation. Participants in their study watched videos of a hand reaching and grasping one of two objects, positioned to the right or left midline, so that trajectory could be independently manipulated. Their results showed a strong RS effect in the left anterior intraparietal sulcus (aIPS) when the same object was grasped, irrespective of the trajectory. Instead, RS effects for trajectory were observed in left lateral occipital sulcus and right PMd cortex. These findings provided a clear evidence for an action perception hierarchy that is based on differences between reach trajectory kinematic parameters and the goal of the action defined by the grasped object. In line with the findings of Hamilton and Grafton, our study confirm that the ventral circuit belonging to the MNS, formed by by the aIPS, IPL and PMv cortex encodes the action goal , irrespective of the kinematic parameters related to its execution (the mean). It is important to note that in our study, the goal was the same in all experimental conditions, but the kinematics of execution was quite different due to intrinsic hand skills of the actors. Indeed, an interesting result was that even if the middle IPS was functionally connected with the SPL region showing a strong modulation caused by changes in the execution ability, the PMv cortex did not show any difference in activation, suggesting a possible functional difference between dorsal and ventral parieto-frontal circuits. This interpretation is also corroborated by a subsequent fMRI study by Hamilton and Grafton (2008) using RS effect to disentangle the contributes of different areas of the MNS in the processing of outcome versus kinematic parameters. An RS effect for outcomes was found in the bilateral IPL and IFG, belonging to the ventral circuit of MNS/AON. Instead, an analysis of the RS effects for the means in which the action was performed, comparing repeated and novel movements irrespective of their outcome, identified an effect in the left SPL, left middle IPS, left occipito-temporal cortex and STS. These results support the idea that visual occipito-temporal areas and dorsal regions of the MNS/AON are involved in the processing of kinematic parameters (i.e. grip, trajectory, means, velocity etc.). Using a different approach based on functional connectivity, we demonstrated that during the observation of actions performed with similar motor skills, the observers tended to resonate stronger compared to the observation of actions beyond the personal own motor repertoire. Our interpretation is that this effect could be supported by the processing of kinematic features within the dorsal network compared to the ventral one, which is more involved in the processing of goals and intention.

Functions of the dorsal MNS/AON: implications for motor learning

The dissociation between ventral and dorsal network within the MNS/AON allow us to hypothesize different involvement of the two modules in the processing of observed and executed actions. Previous studies tried to distinguish the contribute of these parallel networks as modules for reaching and grasping (see Jannerod et al. 1998). In accordance with the notion of motor resonance, observed proximal reaching movements are typically associated with the activation of the dorsal parieto-frontal network linking the convexity of the SPL to the PMd cortex. Only few studies investigated the brain representation of reaching motor acts isolated from the grasp component (Filimon et al. 2007; Malfait et al. 2010; Di Dio et al. 2013). These studies showed activations in a dorsal parieto-premotor circuit spatially overlapping with areas activated during the execution or imagination of reaching. On this basis, one could argue that the findings of our study could be due to aspects of the processing of reaching movements. However, in our study the video-clip displayed object manipulation in a first-person perspective, starting with the hand grasping the object, while an explicit reach component was excluded. Thus, our findings support the involvement of a process that relies on the representation of movement kinematics, irrespective of the goals and the intentions. It is important to stress that the activity of the PMd cortex and SPL region is considered a strong predictor of different kinds of motor learning (Hardwick et al. 2013). In particular, several data indicates that the PMd cortex is an important structure associated with motor learning. In the monkey, PMd has reciprocal connections with the primary motor cortex and direct descending spinal projections (Dum and Strick, 2005). One can argue that the PMd cortex found activated in our study and functionally connected to the SPL may contribute to motor learning at a level higher than pure movement performance. Previous works also indicate that PMd contributes to motor learning by selecting appropriate responses according to visual cues (Kalaska and Crammond, 1995; Picton et al. 2007). Also the SPL region is also considered involved in motor learning. The primate PMd receives afference from the SPL (Matelli et al. 1998) and these regions work in close cooperation to allow visuomotor transformations (Wise et al. 1997). Transforming the sensory input into motor output thus appears to involve a route from the SPL to M1 via the PMd cortex (Johnson et al., 1993, 1996). Our findings derived from regression analysis demonstrate that also in healthy individuals small differences in hand motor dexterity could be associated with different pattern of activations within PMd cortex and SPL region, during the observation of an expert model. Indeed, we found greater activation of the dorsal parieto-premotor network in individuals with higher hand dexterity, compared to individuals with low performances. In principle, this could suggest that during a training based on action observation and imitation, one could have a greater improvement of hand motor function, dependent of his/her baseline motor dexterity level.

From a clinical point of view, these findings enrich the knowledge about the role of different networks in brain reorganization after brain damage extended to the parieto-frontal MNS. In fact, lesion of the posterior parietal cortex leads to severe deficits of goal-oriented movements, known as optic ataxia (Pisella et al. 2000; Karnath and Perenin 2005) which is particular evident when online correction of the movement is required. In addition, lesion to the PMd cortex in humans leads to impairments in the selection among alternative motor schemas (Chouinard and Paus

2006). Consequently, the posterior parietal cortex including the SPL has been related to motor preparation and online correction, while the PMd has been proposed to have a key role in movement selection, in accordance with kinematic laws of motion (Brass and Haggard 2008). In conclusion, it is possible to hypothesize that the dorsal network is involved in the kinematic analysis of motion with respect to the ventral one, suggesting that patients with preserved cortical activity within the dorsal MNS/AON could resonate strongly during the observation of actions performed by a model kinematically similar to their motor experience.

Study 2

Modulating the Mirror Neuron System by Action Observation in Children with Right Unilateral Cerebral Palsy.

3.1 Introduction

Cerebral palsy (CP) refers to a group of permanent disorders of the development of movement and posture, derived from non-progressive disturbances occurring in the developing foetal or infant brain (Graham et al. 2016). Often motor deficits are accompanied by sensory, perceptual, cognitive, communicative and behavioural impairments. Furthermore, CP patients can present epileptic and musculoskeletal problems. CP syndrome is the most common childhood motor disability ranging from 1.5 to 2.5 per 1,000 live births (Krägeloh-Mann and Cans 2009). Children with unilateral cerebral palsy (UCP), in which mostly one side of the body is involved, represent 39% of the general CP population (Shevell et al. 2009; Oskoui et al. 2013). UCP can be attributed to perinatal ischemic stroke or, in premature infants, to white-matter damage producing unilateral porencephalic cavities (or cysts) (Cioni et al. 1999; Graham et al. 2016). In UCP, the upper limb is generally more affected than the lower one (Uvebrant 1988; Cioni et al. 2009), and unimanual activities are normally performed by the unaffected upper limb; daily life activities, which are prevalently bimanual, could be severely impaired (Sköld et al. 2004; Sakzewski et al. 2010).

Several models of intervention are currently available to improve upper limb function in UCP patients, such as intramuscular injections of botulinum toxin A (BoNT-A) alone or combined with upper limb training, constraint-induced movement therapy (CIMT), hand-arm intensive bimanual training (HABIT), and neurodevelopmental treatment (NDT) (Sakzewski et al. 2009). A relatively new rehabilitative approach is based on systematic observation of actions followed by execution (Action Observation Therapy, AOT). The neural model for this therapy is the mirror neuron system (MNS), originally discovered in the monkey premotor and parietal cortex, formed by visuomotor neurons which fire both when a monkey performs a goal-directed motor act or when it simply observes the same or a similar motor act (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996; Fogassi et al. 2005; Rozzi et al. 2008). A comparable MNS has been identified also in humans using several electrophysiological and neuroimaging techniques (Grafton et al. 1996; Grezes et al. 1999; Iacoboni et al. 1999; Buccino et al. 2001; Caspers et al. 2010; Molenberghs et al. 2012). Its two main nodes are the inferior parietal lobule (IPL) and the ventral premotor cortex (PMv), plus the caudal part of the inferior frontal gyrus (IFG) (Rizzolatti et al. 2014). The function of this parieto-frontal network is to transform the visual representations of observed motor acts into the corresponding motor representations, allowing observers to immediately recognize motor acts belonging to their own motor repertoire, that includes not only usual daily life behavior, but also motor skills resulting by long-lasting practice. Indeed, several studies (Buccino et al. 2004a; Calvo-Merino et al. 2005, 2006; Haslinger et al. 2005; Cross et al. 2006; van Elk et al. 2008) indicate the important role of MNS in representing previously acquired motor skills, showing that this system is highly activated during observation of actions already embodied in the observers' motor repertoire. In line with this idea, in the past 10 years an increasing number of AOT applications have been reported in adults and children, using paradigms combining action observation with subsequent execution of observed actions (Ertelt et al. 2007; Celnik et al. 2008; Franceschini et al. 2010, 2012; Sale and Franceschini 2012; Brunner et al. 2014; Buccino 2014; Sarasso et al. 2015; Sugg et al. 2015). Recently, AOT efficacy has also been investigated in UCP children (Sgandurra et al. 2013) showing significant motor improvement after three-week training period.

In AOT of UCP children it is possible that observation elicits, in the observer, a marked propensity to preserve task proficiency by selecting movements that enable attainment of the action goal, regardless of kinematic resemblance with observed model. This could be because the complexity of the model offered during observation of an action performed by a healthy hand is not appropriate for imitation, so that children decide to copy the final outcome of the action rather than the process used to achieve it. This could also explain why in daily life UCP children do not simply imitate their unaffected hand. Thus, given the paucity of their motor repertoire with respect to healthy children, CP children might have a reduced activation of the MNS during observation of actions performed by healthy subjects. Nonetheless, we hypothesize that this activation might increase during observation of the same actions performed by a child with comparable coping strategies. Hence, on the assumption that motor experience is able to modulate activation of MNS, we performed a functional Magnetic Resonance Imaging (fMRI) study on UCP and typically developing (TD) children, to verify the neural correlates of the observation of actions performed by agents with two different levels of motor skills, namely a UCP child with a moderate degree of hand impairment and an agent with a healthy hand. We hypothesized that in UCP children the observation of actions performed with a paretic hand should be more effective in producing MNS activation than the observation of a healthy hand.

3.2 Materials and methods

3.2.1 Participants

Ten children (see Table 5) with unilateral brain lesion and spastic UCP (7 males and 3 females; range 9-14 years; M = 11.2; SD = 2.09) were selected from hemiplegic children database of IRCCS S. Maria Nuova Hospital (Reggio Emilia, Italy) according to the following inclusion criteria: 1) confirmed diagnosis of right spastic UCP according to definition (MRI and clinical history); 2) age 9-14 at time of recruitment; 3) mild or moderate upper limb disability, i.e. active use of affected upper limb ranging from poor active assisted use to spontaneous use, according to House Functional Classification System (HFC, House et al. 1981) with grades between 4 and 5 (corresponding to *synergic hand* of Ferrari and Cioni Kinematic Hand Classification (Ferrari and Cioni 2009; see Table 4); 4) no cognitive, visual or auditory impairments; 5) no history of seizures or seizures well controlled by therapy; 6) sufficient cooperation in performing 45-minutes fMRI session.

Children were excluded if they: 1) received BoNT-A injections or had upper limb surgery within 6 months prior to testing; 2) were unsuitable for 3 Tesla Magnetic Resonance System due to metal implants, shunts, etc.; 3) were ranked < 4 according to HFC; 4) had moderate or severe muscle spasticity and/or contracture (MAS > 2) (Bohannon and Smith 1987) so requiring spasticity management or orthoses. Ten right-handed (Oldfield 1971) TD children were enrolled as control group (5 males and 5 females; range 9-14 years; M = 12.4; SD = 1.2). All TD and UCP children attended regular public school and could consistently follow instructions. Children who met all inclusion criteria were invited to participate in this fMRI study and informed consent was obtained from children and/or parents prior to recruitment, according to the Declaration of Helsinki. The experimental procedure was approved by the Local Ethics Committee (University of Parma).

Grasping modality	Simple synergy of grasping triggered through controllable elbow and shoulder movements and under visual control. No hand adaptation to object. Grasping is possible only if unaffected hand takes part in the same action.
Reaching	Orientation, anticipation and pre-adaptation with insufficient motor planning modulation. Reaching dependent on flexion and extension synergies.
Visual-motor integration	The object is passed with difficulties from the affected hand to unaffected one, better vice versa. Necessity of visual control.
Releasing	The object is released slowly and with difficulty, often using servomotor movements.
Manipulation	Extremely limited. The manipulation is possible only if the object possesses suitable dimension, weight and consistency.
Bimanual activity	Possible collaboration in achieving the same aim. Affected hand is used to support the unaffected one. There is no part of lateral hemispace in which the affected hand can be spontaneously utilised first.
Main core	Stereotypically expressed grasping and releasing within flexion and extension synergies.

Table 4: Description of kinematic and functional features of *Synergic* hand in UCP children.

3.2.2 Clinical Assessment

UCP children participating in this study were assessed using the HFC (House et al. 1981; Koman et al. 2008), Kinematic Classification System (Ferrari and Cioni 2009) and Manual Ability Classification System (MACS; Eliasson et al. 2006). HFC is reliable tool for assessing upper extremity function in children with CP. It was developed for the evaluation of function in the affected hand after surgery for thumb-in-palm deformity in children with spastic hemiplegic CP (House et al. 1981) and has been used to evaluate children before and after upper extremity BoNT-A injections (Hoare et al. 2010). The classification consists of 9 grades ranging from a hand that is not used at all (grade 0) to one that is used spontaneously and independently from the other hand (grade 8). The HFC has been reported to have an excellent interrater (Intraclass Correlation Coefficient, ICC = 0.92) and intrarater reliability (ICC = 0.94) (Koman et al. 2008). Ferrari and Cioni Kinematic Classification describes five patterns of manipulation in hemiplegic children by analyzing hand kinematic profile and functional use (Ferrari and Cioni, 2009). According to it, all ten selected patients had a *synergic hand* (Table 4). The MACS consists of five levels and allow classifying how children with CP aged 4 to 18 years use their hands when handling objects in daily activities (Eliasson et al. 2006). Individual scores for these classification scales are reported in Table 5.

Subject #	Sex	Age	Lesion side	GA (wks)	CP Type	GMD	WMD	Radiological findings	HFC	MACS	KHC
1	M	9	LH	26	Right UCP	-	x	Periventricular malacic areas with mild VD	5	2	Synergic hand
2	F	11	LH	25	Right UCP	x	xx	Moderate CC atrophy; parietal periventricular malacic area with moderate VD; fronto-parietal gliosis; parietal cortical atrophy	5	3	Synergic hand
3	F	13	LH	32	Right UCP	x	x	Moderate CC atrophy; fronto-periventricular malacic area with moderate lateral VD	5	3	Synergic hand
4	M	14	LH	38	Right UCP	-	xxx	Severe CC atrophy; fronto-parietal periventricular and subcortical malacic area with moderate lateral VD; interemispheric cyst	5	3	Synergic hand
5	M	9	LH	32	Right UCP	xx	xx	Severe CC atrophy; fronto-insular periventricular and subcortical malacic area with severe lateral VD; parietal WM gliosis; Wallerian degeneration; posterior fossa arachnoid cyst	5	3	Synergic hand
6	M	9	LH	40	Right UCP	-	x	Parietal periventicular malacic area with lateral VD; frontal gliosis; CC hypotrophy	5	3	Synergic hand
7	F	13	LH	36	Right UCP	xx	xx	CC hypotrophy; fronto-parietal insular periventricular and subcortical malacic area with lateral VD; Wallerian degeneration	4	3	Synergic hand
8	M	10	LH	37	Right UCP	xx	x	Mild posterior CC hypotrophy; parietal periventricular and subcortical malacic area with mild lateral VD	5	3	Synergic hand
9	M	10	LH	30	Right UCP	-	x	Parietal periventricular malacic area with minimal lateral VD	5	3	Synergic hand
10	M	14	LH	36	Right UCP	-	xx	Severe CC athrophy; fronto-parietal periventricular malacic area with severe lateral VD	5	3	Synergic hand

Table 5. Demographic data, clinical features, radiological findings and functional classification in cases group. CC = Corpus Callosum; F = Female; CP = Cerebral Palsy; LH = Left Hemisphere; GA = Gestational Age; GMD = Grey Matter Damage; HFC = House Functional Classification; KHC = Kinematic Hand Classification; M = Male; MACS = Manual Ability Classification System; VD = Ventricles Dilatation; WMD = White Matter Damage; x = mild damage; xx = moderate damage; xxx = severe damage.

3.2.3 Experimental Design, Stimuli and Tasks

Action Observation task

A set of 48 videos showing grasping actions executed by the right hand, each lasting 4 seconds, was used in this study. These actions consisted in grasping a small object (a sphere, a cube, a cylinder) and putting it into one of two square boxes (size 6 x 6 cm) placed on the left and the right side with respect to the object. An example of the stimuli used in the action observation task is shown in Figure 17 A.

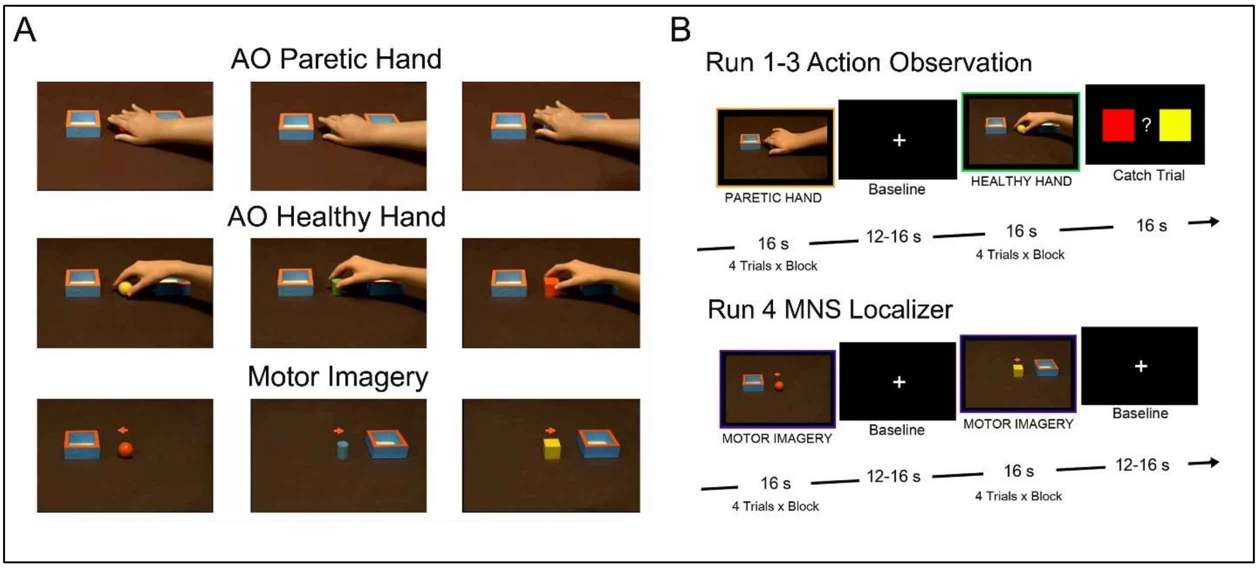


Figure 17: Experimental stimuli and task design. (A) In the action observation runs, participants observed videos showing actions performed by a paretic (AO Paretic Hand) or an healthy hand (AO Healthy Hand), from a first person perspective; actions consisted in reaching-grasping an object (a sphere, a cube, a cylinder) and putting it into one of two boxes. During MNS Localizer, participants were asked to observe a video showing an object and a box on a table and simultaneously imagine themselves grasping the object with the right hand and placing it in the box. (B) Action observation task was presented in 3 functional runs, each made up of 16-s independent blocks, consisting of four randomly presented videos of the same condition. Each block was interleaved by a *Rest* period (12-16-s). In 25% of action observation blocks, participants had to indicate the color of the presented object or the box (left or right) in which the object was placed. The MNS Localizer was composed by 8 blocks, each formed by 4 trials, lasting 4-s.

The object color was either red, yellow, green or blue. All videos were depicted in a first-person perspective, as if the observer was performing the action. There were two video conditions: a) actions performed by an actor without any motor impairment (Action Observation Healthy Hand – *AO Healthy Hand*); b) actions performed by a UCP patient with a moderate level of hand impairment, corresponding to *synergic hand* of Kinematic Hand Classification (Action Observation Paretic Hand – *AO Paretic Hand*). All actions were performed with the same visual context, balanced for luminance, color and amount of visual information. The action observation task was presented in 3 functional runs, each made up of 16-s independent blocks (Figure 17 B), consisting of four randomly presented videos of the same condition. Each functional run was composed by 6 blocks for each condition. Each block was interleaved by an inter-blocks *Rest* period (12-16 s) during which no video was presented. In the action observation task, participants were instructed to carefully observe the presented action paying attention to it. A similar paradigm has been used in previous action observation studies in adults and children (see Biagi et al. 2016). In 25% of blocks, catch trials were randomly presented in which participants had to indicate the color of the presented object or the box (left or right) in which the object was placed, by pressing, with the unaffected hand, a two-choice button on a response pad placed inside the scanner. This response was unrelated to the motor content of presented video. A total of 48 experimental video stimuli (3 objects x 4 colors x 2 object positioning x 2 conditions) were presented in the action observation task.

MNS Localizer

In order to identify neural regions involved both in action observation and execution, participants performed a MNS Localizer at the end of scanning session. Since executing a real action inside the scanner was not feasible for UCP patients, due to excessive head motion and difficulty in minimizing mirror movements in the unaffected hand, during the Localizer our participants performed an explicit motor imagery task (see e.g. Filimon et al. 2007). Participants were presented with videos showing an object (sphere, cube or cylinder) and a box (6 x 6 cm) on a table (Figure 17 B). They were instructed to observe the presented setting, then a cue (a little arrow appearing in the central part of the screen) asked them to imagine performing the action with their right hand (e.g. imagine grasping the object and placing it in the box located in the position cued by the arrow). An example of the stimuli used in the MNS Localizer is shown in the Figure 17 A. A total of 24 experimental video stimuli (3 objects x 4 colors x 2 box location) were presented in 8 blocks of the MNS Localizer. Each block was formed by 4 trials, each lasting 4 s.

3.2.4 Scanning procedure

Participants went through a training phase before each fMRI session aimed at familiarizing them with the experimental procedure. During scanning, participants laid supine in the bore of the scanner. Visual stimuli were presented in the fronto-parallel plane by means of a digital video system (60 Hz refresh rate) with a resolution of 800 horizontal pixels x 600 vertical pixels with horizontal eye field of 30° (Resonance Technology, Northridge, CA). Sound-attenuating headphones were used to muffle scanner noise and give instructions to subjects. Digital transmission of signal to scanner was via optic fiber. Software E-Prime 2 Professional (Psychology Software Tools, Inc., http://www.pstnet.com) was used both for stimulus presentation and for recording of participant response. The action observation task was arranged in a block-design, in which all conditions (*AO Paretic Hand*, *AO Healthy Hand*, *Rest*) were evenly distributed across 3 runs, which lasted 936 s (234 TRs). The MNS Localizer run utilized a block-design with 16-s motor imagery blocks, which were interspaced with 12-16-s *Rests* and lasted 528 s (132 TRs). Before beginning of MRI acquisition, subjects received the instruction not to make any voluntary movement during the action observation and MNS Localizer and only concentrate on the video screen. Absence of actual hand movement during tasks was visually checked by the investigator. The experiment was composed by 4 functional runs, 3 runs for the action observation task (with a total of 18 blocks and 72 single trials for each condition, presented in a randomized order) and 1 run for the MNS Localizer (with a total of 8 blocks and 32 single trials). Each of the action observation runs lasted about 8 min. The MNS Localizer run lasted about 5 min.

3.2.5 Magnetic resonance imaging data acquisition

Anatomical T1-weighted and functional T2*-weighted MR images were acquired with a 3-T General Electric scanner equipped with an 8-channel receiver head-coil. A three-dimensional (3D) high-resolution T1-weighted IR-prepared fast SPGR (Bravo) image covering the entire brain was acquired in one of the scanning sessions and used for anatomical reference. Its acquisition parameters were as follows: 196 slices, 280×280 matrix with a spatial resolution of 1×1×1 mm, TR = 9700 ms, TE = 4 ms, FOV = 252 x 252 mm; flip angle = 9°. Functional volumes were acquired while participants performed the action observation task and the MNS Localizer with the following parameters: thirty-seven axial slices of functional images covering the whole brain acquired using a gradient-echo echo-planar imaging (EPI) pulse sequence, slice thickness = 3 plus interslice gap = 0.5 mm, 64×64×37 matrix with a spatial resolution of 3.5×3.5×3.5 mm, TR = 2000 ms, TE = 30 ms, FOV = 205 x 205 mm^2, flip angle = 90°, in plane resolution = 3.2 x 3.2 mm^2.

3.2.6 fMRI data processing and analysis

Data analysis was performed with SPM8 (Wellcome Department of Imaging Neuroscience, University College, London, UK; http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2016 (The Mathworks, Inc.). Structural images were manually centered and reoriented with functional images to the anterior-posterior commissure axis. The first four EPI volumes of each functional run were discarded to allow for T_1 equilibration effects. For each subject, all volumes were slice timing corrected, spatially realigned to the first volume of the first functional run and un-warped to correct for between-scan motion. Motion parameters were used as regressors of no interest in the model to account for translation and rotation along the 3 possible dimensions as determined during the realignment procedure. Individual dataset was excluded if excessive head motion was observed (translation > 3 mm or rotation > 3°). T_1-weighted image was segmented into gray, white and cerebrospinal fluid and spatially normalized to the Montreal Neurological Institute (MNI) space. Spatial transformation derived from this segmentation was then applied to the realigned EPIs for normalization and re-sampled in 2×2×2 mm^3 voxels using trilinear interpolation in space. All functional volumes were then spatially smoothed with a 6-mm full-width half-maximum isotropic Gaussian kernel.

Univariate Statistical Analysis

The pre-processed functional data for each participant were entered in single-subject whole-brain analysis (Friston et al. 1995). Blood oxygen level dependent (BOLD) signal was modeled in a General Linear Model (GLM) by a design matrix comprising onsets and durations of each event according to the experimental task for each functional run. This analysis employed event-related convolution models using the hemodynamic response function (HRF) provided by SPM8. In single subject, we assessed BOLD signal changes with the following contrasts: Observation of actions performed with the paretic hand vs. baseline (*AO Paretic Hand* vs. *Rest*); Observation of actions performed with the healthy hand vs. baseline (*AO Healthy Hand* vs. *Rest*). For the MNS Localizer we used one predictor of interest that is a boxcar function with the duration of the motor imagery blocks, containing 4 trials each. Single-subject analysis was performed with a fixed-effect approach (FFX), using a statistical threshold of $P < 0.001$ for action observation task and $P < 0.005$ for MNS Localizer, after correction for multiple comparison using the family-wise error (FWE) rate at the cluster level, in order to replicate, in each participant, activation maps found in their respective group. In order to assess the general activation pattern in UCP children and controls, two statistical approaches were used. First, a standard whole-brain analysis was performed using a random effects model (Friston et al. 1999), implemented in a second-level procedure (See Figure 20). Second-level statistical parametric maps were interpreted using a statistical threshold of $P < 0.001$, applying a FWE correction rate for multiple comparison at cluster level. However, for clinical fMRI studies, standard GLM analysis is not always relevant on an individual patient level, due to the lesions extension. For these reasons, we computed consistency activation maps using a voxel-based overlap method (Seghier and Price 2016). These functional overlap maps complement standard (random) group analyses by indicating how consistently a given effect occurs across subjects. The range of percentage used for our maps was from 40 to 100% (meaning that a voxel appeared in the maps only if it was activated in at least four subjects, and that the maximum value of the color scale was given by areas activated in all ten subjects belonging to the same group). Anatomic descriptions were performed on the basis of probabilistic cytoarchitectonic maps as implemented in the SPM8 Anatomy toolbox (http://www.fz-juelich.de; Eickhoff et al. 2005).

Region of interest (ROI) analyses

In order to investigate possible different responses to actions performed with a paretic or healthy hand (motor experience effect) we performed a ROI analysis confined to two important MNS areas, premotor cortex (PMC) and posterior parietal cortex (PPC). Because in humans and brain damaged children determining a precise localization for these ROIs is more difficult than in monkeys, we established our ROIs at a single-subject level on the basis of functional properties. Specifically, our ROIs were identified by means of the results obtained for the MNS Localizer based on an explicit motor imagery task. ROI analysis included the two within-subject factors

(*AO Paretic Hand*, *AO Healthy Hand*) and was performed on the mean percent signal change (PSC) extracted using the SPM Rex Toolbox (http://web.mit.edu/swg/software.htm) for each UCP and TD participant from the local maxima of the single-subject activation map. Our ROIs (10 mm spheres) were described macro-anatomically (i.e., premotor cortex, PMC; posterior parietal cortex, PPC). PSC data were analyzed with a *t*-test to determine direction of each significant main effect, applying a statistical threshold of $P < 0.05$. Furthermore, we plotted event-related responses for each participant and for each ROI using MarsBar SPM Toolbox. This graph showed adjusted data and fitted response across peri-stimulus time, both at a single-subject level and for multi-subjects analysis.

Testing for task attention: Behavioral Analysis

To test the response accuracy of participants, we carried out a further analysis based on the responses given by children during scanning sessions when catch trials were presented, that is, those trials in which the participants were required to give an explicit response based on presented stimuli. More specifically, during the action observation task, participants had to indicate the color of presented object or box in which the object was placed, by pressing a button on a response pad placed inside the scanner. For each participant, 12 responses were recorded during the action observation task (4 for session).

Lesion Analysis

Lesions were manually delineated on the T_1-weighted images using MRIcro Toolbox (http://www.cabi.gatech.edu/mricro/mricro). Individual lesions were mapped by an expert radiologist delineating the boundary of the lesion directly on the image for every single transverse slice using MRIcro software. Both the MRI scan and the lesion shape were then mapped into stereotaxic space using a normalization algorithm provided by SPM8. After normalization, all lesions were carefully reviewed to ensure that lesion maps accurately reflected the extent of lesions in MNI space. Manual adjustments were made if necessary. Lesion overlap was computed using MRIcro software.

3.3 Results

Lesion Anatomy

Figure 18 shows an overlap of the lesions of all UCP children and the 3D individual lesion reconstruction. Most lesioned regions involved the periventricular area. The highest lesion overlap was found in subcortical white matter of left hemisphere. Conversely, cortical involvement of regions outside the periventricular zone, i.e. inferior frontal, dorsolateral frontal, inferior and superior parietal regions, was much less frequently found (N = 3 subjects). Overall, lesion distribution was similar to previous CP lesion studies (Dinomais et al. 2013a; Van de Winckel et al. 2013).

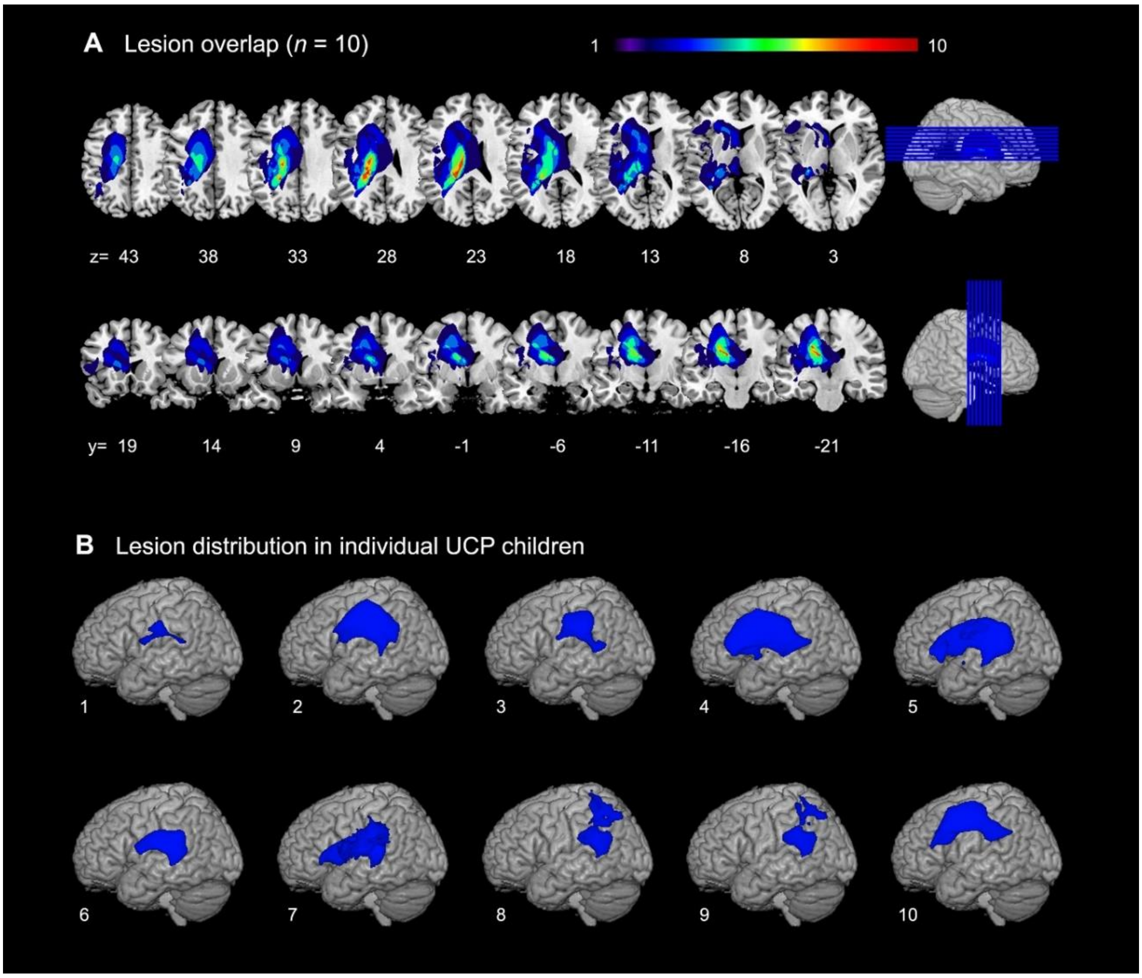


Figure 18: Overlap of the binarized lesion maps of the 10 UCP patients included in the study. (A) The color bar indicates the degree of overlap of lesions, e.g. blue color indicate that in 3 of 10 subjects, tissue was lesioned, while red color indicate the maximum overlap. (B) Illustration of the lesions of the individual patients. Lesions are projected onto the surface of the brain regardless of their depth (maximum intensity projection).

Behavioral responses during functional MRI

To test the responses accuracy, we carried out an analysis based on the responses given by the participants during the scanning sessions when presented with the catch trials. Mean response accuracy recorded during catch trials was 85.6% (SD = 10.4%) for UCP children and 89.2% (SD = 8.4%) for TD children. There were no statistical differences between TD and UCP children for the numbers of corrected answers or response latencies.

Functional MRI results

Voxel-based overlap maps

Figure 19 illustrates voxel-based overlap maps for the UCP and TD groups during action observation, based on the contrasts *AO Paretic Hand* vs. *Rest* and *AO Healthy Hand* vs. *Rest*. In UCP, activation patterns were largely symmetrical, although some clusters were only present in the right contralesional hemisphere, such as PMC activation, during observation of the healthy model. During observation of both paretic and healthy hand, consistent voxels across the 10 patients were detected in areas belonging to the MNS and the so-called Action Observation Network (AON, Caspers et al. 2010), including occipito-temporal, posterior parietal and premotor cortices. Occipito-temporal activation reached a maximum near the middle temporal gyrus (MT/V5). This activation shows two rostrally-directed branches: a dorsal one extending to the posterior superior temporal gyrus (pSTG), and a ventral one extending into the posterior occipito-temporal sulcus (pOTS). Furthermore, there was an increased activation in the PPC, including both inferior (IPL) and superior parietal lobule (SPL). A large premotor activation, more lateralized to the right contralesional hemisphere, was observed during both action observation conditions.

In TD children, in accordance with studies on action observation in adults, activation maps were more lateralized to the left hemisphere, as also evidenced in the second-level group analysis (Figure 20). Activation was observed in areas belonging to AON, such as the superior/middle temporal gyrus (STG, MTG), anterior intraparietal sulcus (aIPS) and IPL, SPL and PMC. All activations at single subject level was thresholded at $P < 0.001$, after correction for multiple comparison (FWE) at the cluster level.

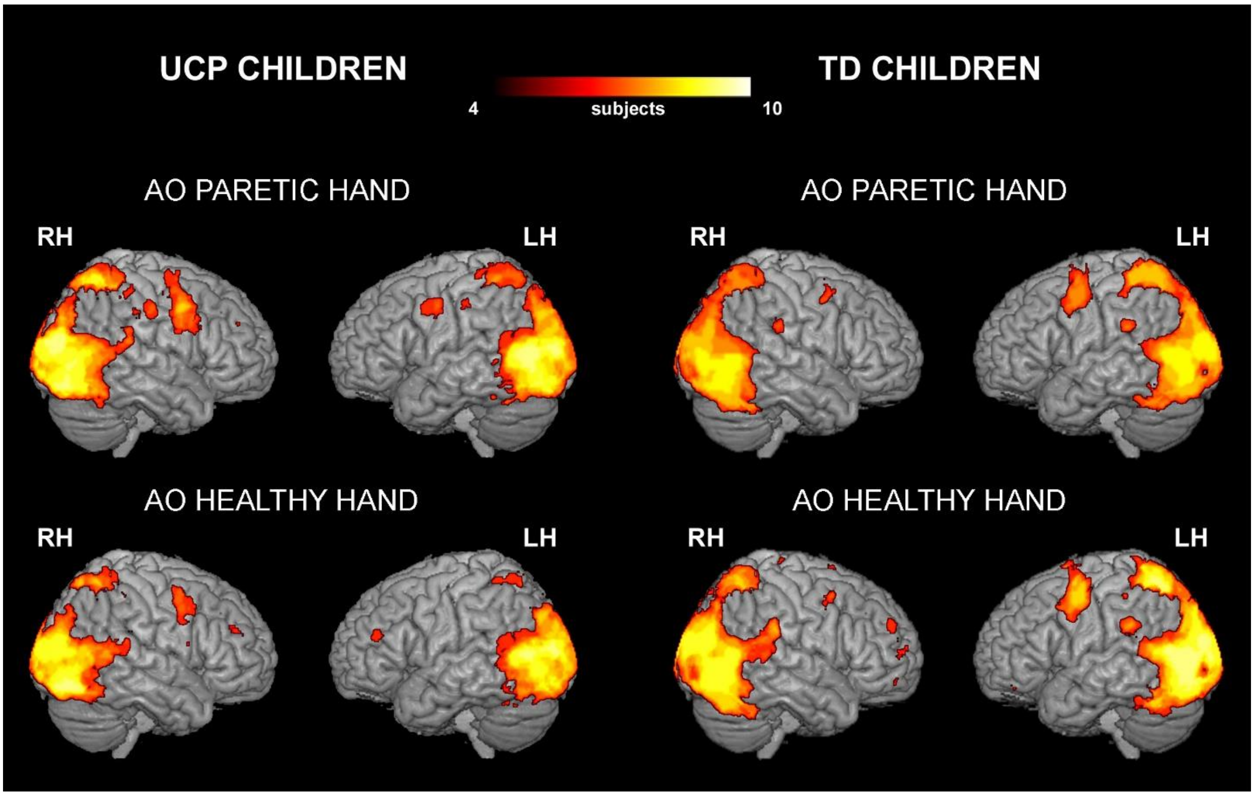


Figure 19: Voxel-based overlap maps of the MNS in UCP and TD children. Each panel shows a different contrast: *AO Paretic Hand* vs. *Rest* in UCP (top-left panel), *AO Paretic Hand* vs. *Rest* in TD (top-right panel), *AO Healthy Hand* vs. *Rest* in UCP (down-left panel), *AO Healthy Hand* vs. *Rest* in TD (down-right panel). Color bar indicates how consistently a given effect occurs across subjects, from 40 to 100% (meaning that the maximum value of the color scale was given by areas consistently activated in all ten subjects belonging to the same group).

Standard GLM Group-Analysis

In Figure 20, activation maps related to action observation are overlaid on the mean images of the UCP and TD groups respectively. The *t*-contrasts testing *AO Paretic Hand* vs. *Rest* and *AO Healthy Hand* vs. *Rest* in both groups, showed enhanced activation within AON areas (for MNI coordinates see Supplementary Tables 6-7). Consistent with spatial-overlap analysis, significant different activation was evident bilaterally for the UCP group and more lateralized in the left hemisphere for TD children, within both dorsal and ventral sectors of the PMC. Furthermore, for TD children, bilateral activation was evident in the parietal cortex within SPL and IPL and IPS. However, the second-level GLM analysis showed for the UCP group enhanced activation during observation of the pathological model in left (lesioned) IPL and SPL, as well as in the PMC. In contrast, during observation of the healthy model, GLM group analysis does not allow to observe significant clusters of activation in the PPC or PMC of left hemisphere of UCP children.

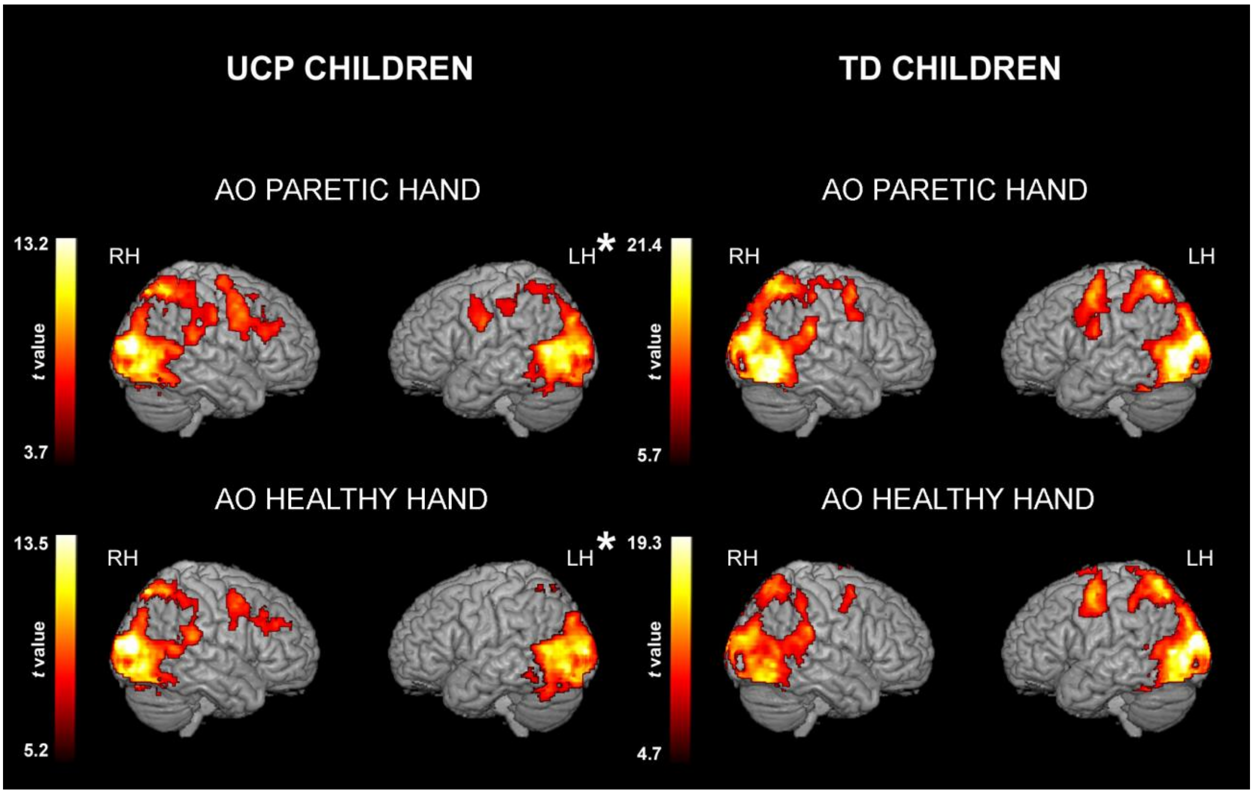


Figure 20: Brain activations resulting from the conditions *AO Paretic Hand* vs. *Rest* and *AO Healthy Hand* vs. *Rest* for both UCP and TD children. These activations are rendered into a standard Montreal Neurological Institute brain template ($P_{FWE} < 0.001$ at cluster level). RH, right hemisphere; LH, left hemisphere. *Lesioned hemisphere.

MNS Localizer

In order to restrict our analyses to the main nodes of MNS, we identified, at a single-subject level, brain areas involved during an explicit motor imagery task (Localizer). Similar to previous studies in adults (see Hetu et al. 2012), imagining of reaching, grasping and placing an object activated, in TD children, the SPL and IPS, the precuneus, as well as the dorsal PMC (superior frontal gyrus/sulcus).

Specifically, in the frontal lobes, bilateral IFG (including the pars opercularis), PMC, middle frontal gyrus (MfG), and the SMA were consistently activated. In the parietal lobes, bilateral SPL and rostral-left IPL were activated. Consistently activated subcortical regions included left putamen, pallidum and right thalamus. Finally, lobules VI (bilateral) of the cerebellum (CB) were found to be consistently activated (Figure 22). Motor imagery in UCP children showed consistent activations in clusters similar to those of TD children (see below ROI Analysis, Figure 4). In the parietal lobes, activations were consistently found in bilateral SPL, IPL, and precuneus, in addition to right postcentral gyrus (PocG). Consistent activations were also found in the CB bilaterally and in left putamen and right pallidum. However, a significant functional lateralization was found during motor imagery in the UCP sample, in agreement with the action observation task (see Figure 21).

ROI Analysis

A ROI-based analysis was performed on the mean PSC, aimed at revealing the specificity for observation of actions performed by the paretic or healthy model. Furthermore, ROI analysis allows testing of BOLD responses at single-subject level, with a high level of accuracy, also considering the lesioned tissue. On the basis of specific lesions and functional reorganization in the UCP sample, we reasoned that testing the effect of a mean entire ROI (averaged on 10 local maxima) might not be representative of the real effects occurring in a given region. PPC and PMC ROIs were centered at single-subject level on the local maxima at $P < 0.001$, after correction for multiple comparisons, in the left hemisphere for all TD participants and in the left (subject #2, #3, #4, #9, #10) or right (subject #1, #5, #6, #7, #8) hemisphere for UCP children, on the basis of the functional reorganization, as shown by MNS Localizer based on motor imagery. Individual MNI coordinates of the local maxima for each ROI in PMC and PPC are reported in Figure 21 for the UCP group and in Figure 22 for the TD group. The averaged hemodynamic response and relative fitted event-related time courses for both experimental conditions were analyzed for both groups in the PMC and PPC ROIs. ROI analysis on the parietal activation showed that in UCP children this region was more strongly activated following observation of paretic hand with respect to healthy one ($P < 0.001$) (Figure 23, A1, A2, A3). This finding emerges from the observation of both the average PSC in right or left hemisphere, on the basis of specific functional reorganization in each UCP child. Similar results were obtained in the PMC region (Figure 23, B1, B2, B3), showing a greater activation for the observation of the pathological model with respect to the healthy one ($P < 0.01$).

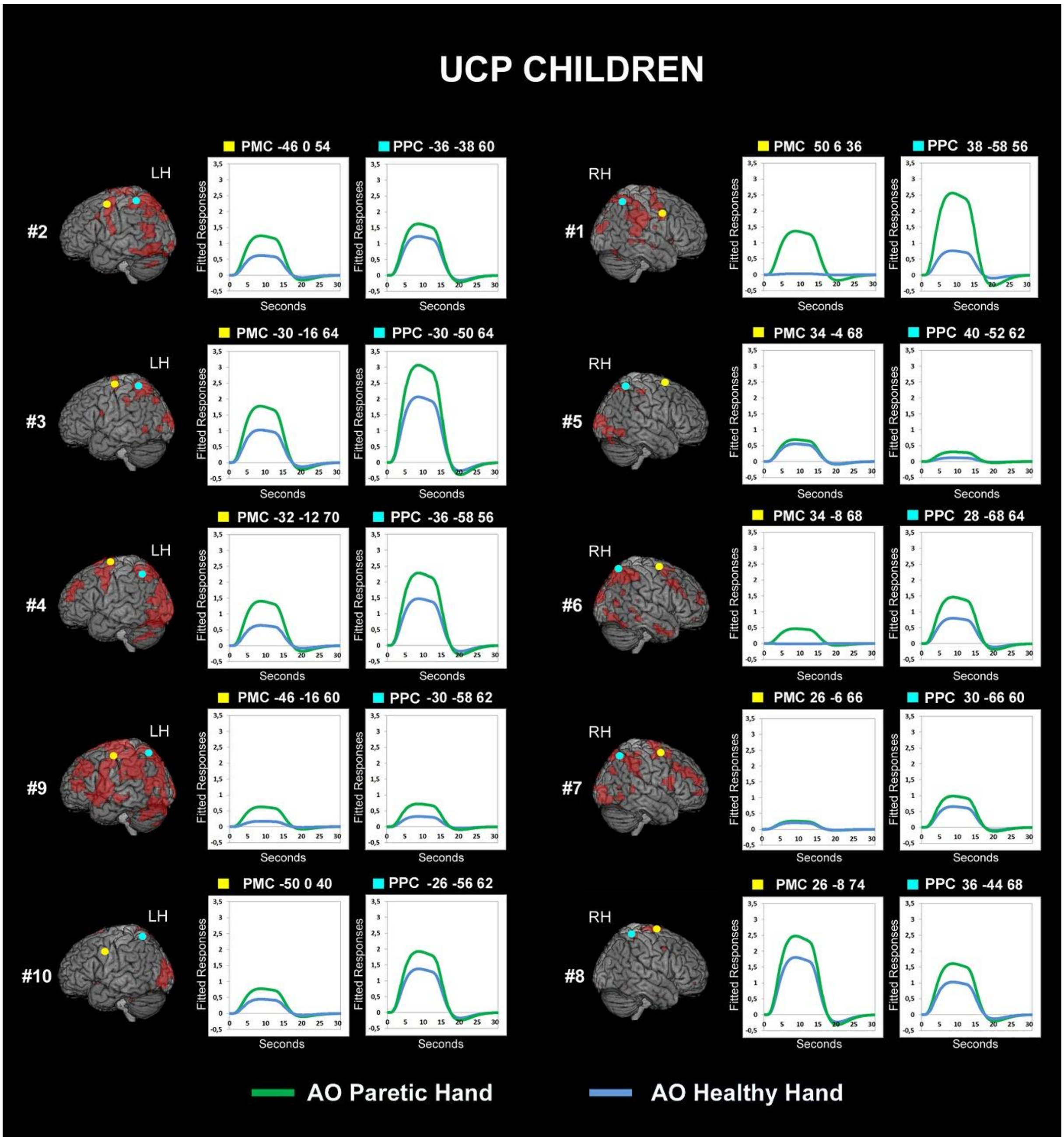


Figure 21: Individual anatomical location and fitted responses for premotor and parietal regions of interest (ROIs) in UCP patients. PMC and PPC ROIs are over imposed on the activation map (red color) resulting from the MNS Localizer based on motor imagery task, displayed into a standard Montreal Neurological Institute brain template ($P_{FWE} < 0.005$ at cluster level). Yellow points indicates peaks of activation within the PMC region, while light blue points indicate peaks in the PPC region, for each individual subject. Plots illustrate individual time course of fitted event-related responses from the conditions *AO Paretic Hand* vs. *Rest* (green line) and *AO Healthy Hand* vs. *Rest* (blue line). LH = Left Hemisphere, RH = Right Hemisphere.

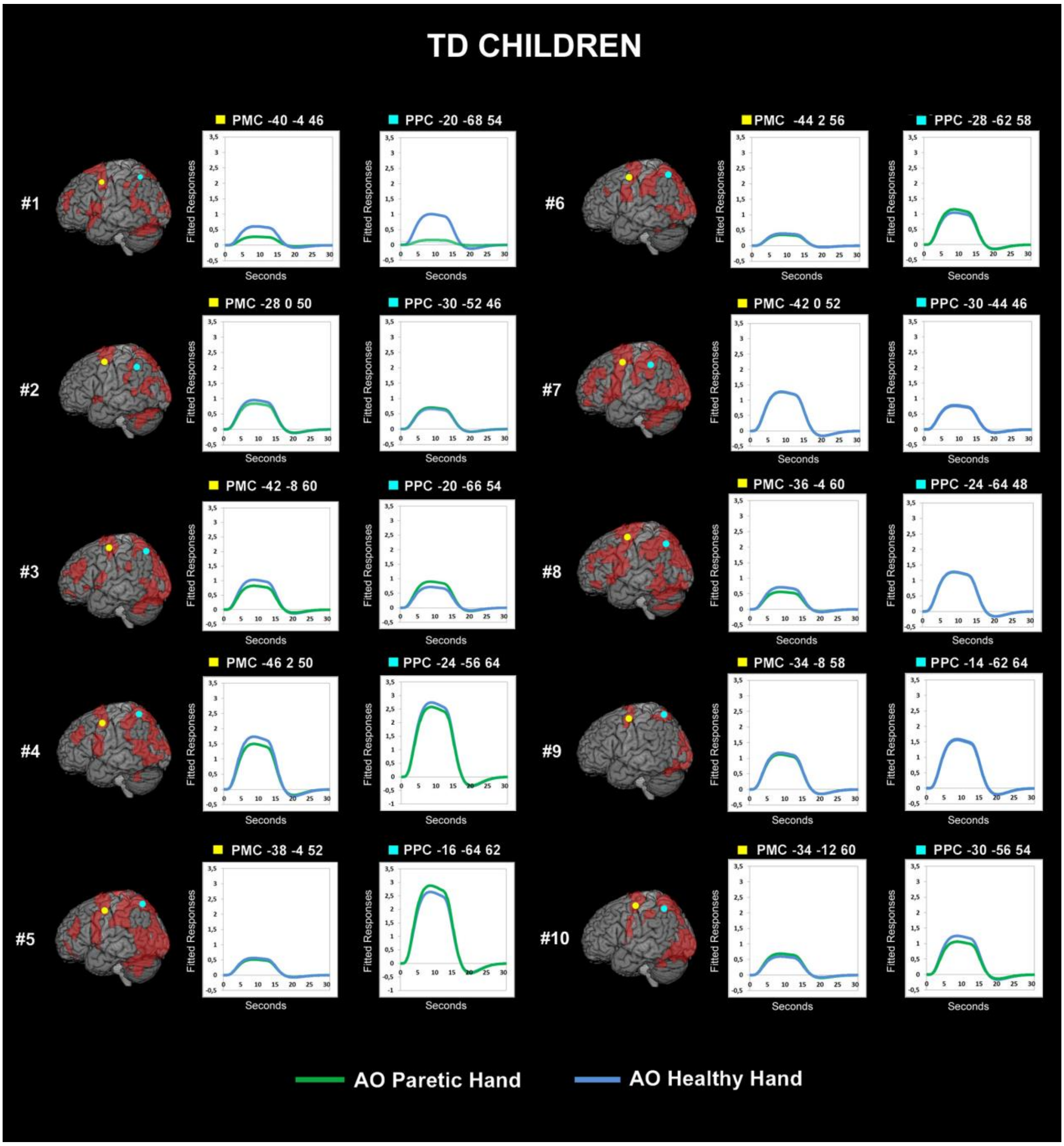


Figure 22: Individual anatomical location and fitted responses for left premotor and parietal regions of interest (ROIs) in TD controls. PMC and PPC ROIs are overimposed on the activation map (red color) resulting from the MNS Localizer, displayed into a standard Montreal Neurological Institute brain template ($P_{FWE} < 0.005$ at cluster level). Yellow points indicates peaks of activation within the PMC region, while light blue points indicate peaks in the PPC region, for each individual TD child. Plots illustrate individual time course of fitted event-related responses from the conditions *AO Paretic Hand* vs. *Rest* (green line) and *AO Healthy Hand* vs. *Rest* (blue line).

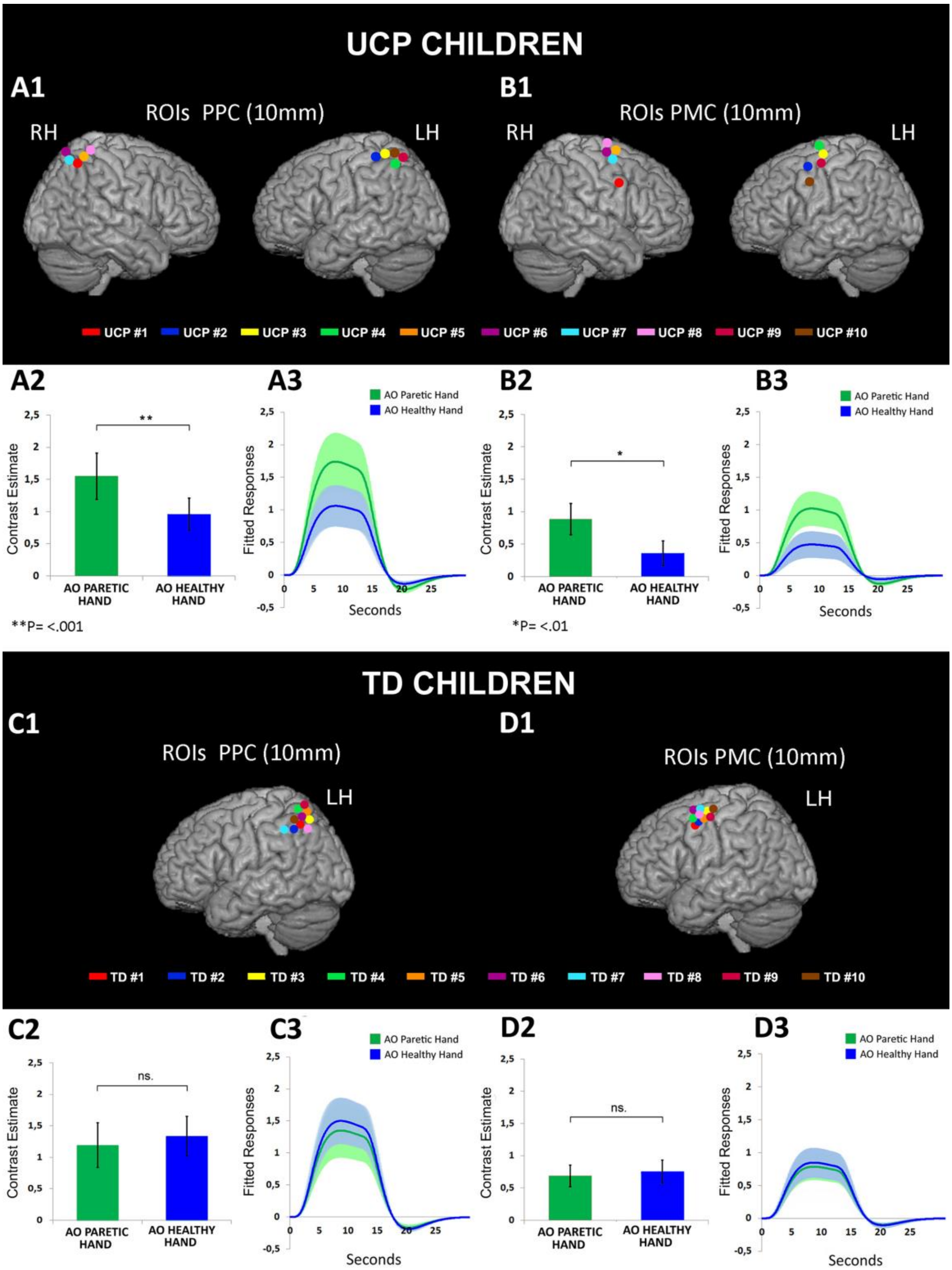


Figure 23: Multi-subjects ROI analysis. Individual anatomical locations of PPC and PMC ROIs displayed into a standard Montreal Neurological Institute brain template, for both UCP patients (A1, B1) and TD children (C1, D1). Histograms show the averaged magnitude of activation (parameter estimate) in each ROI for UCP (A2, B2) and TD group (C2, D2). Line graphs indicate event-related fitted response across peri-stimulus time in PPC and PMC ROIs for the experimental conditions *AO Paretic Hand* (green line) and *AO Healthy Hand* (blue line), in UCP cases (A3, B3) and TD group (C3, D3). Brackets above

the columns indicate the comparison among the conditions *AO Paretic Hand* and *AO Healthy Hand*. Bars and colored area indicate SEM. Asterisks indicate significant differences set at $P < 0.01$ (*) and $P < 0.001$ (**). LH = Left Hemisphere, RH = Right Hemisphere.

On the contrary, in the TD group, BOLD responses in PPC and PMC regions of left hemisphere for observation of paretic vs. healthy hand did not show any significant difference ($P < 1$, n. s.) (Figure 23, C1, C2, C3 for PPC ROIs; Figure 23, D1, D2, D3 for PMC ROIs).

3.4 Discussion

The Study 2 demonstrates that children affected by UCP exhibit enhanced activation of the MNS during observation of goal-directed actions performed by a paretic hand, a model similar to their own motor repertoire, with respect to observation of a healthy hand. Using a ROI-based approach, we found, at both individual and multi-subject level, that activation of the main nodes of MNS in UCP depends on the specific model observed. On the contrary, TD children similarly activate MNS in both observation conditions.

Motor resonance induced by action observation in adults and TD children

Our findings show, in both TD and UCP children, an activation of the parieto-premotor MNS/AON during action observation, confirming and extending previous studies on adults (Grafton et al. 1996; Grezes et al. 1999; Iacoboni et al. 1999; Buccino et al. 2001; Caspers et al. 2010; Molenberghs et al. 2012). Most importantly, they advance the knowledge on development and properties of the MNS in children (see for review Burzi et al. 2015). Some neuroimaging studies showed activation of MNS in TD children and adolescents for different tasks (observation of hands grasping objects, Ohnishi et al. 2004; observing facial emotional expressions, Dapretto et al. 2006; imitation and observation of animated fingers, Williams et al. 2006; observation of neutral and angry hand and facial actions, Shaw et al. 2011). Evidence of a neural response to action observation at 6 and 8 months of age comes also from studies carried out by Nyström and coworkers who used EEG techniques (Nyström, 2008; Nyström et al. 2011). They found a significantly higher ERP amplitude for goal-directed grasping and a significantly greater response to goal-directed actions by measuring μ-desynchronization. In a recent fMRI study, Biagi and coworkers (2016) investigated activation of areas belonging to the MNS in children and adults during observation of complex hand-grasping actions, as compared to observation of simple grasping actions executed by the left and right hand, seen from a first-person perspective. In line with the results of our study, authors showed that in children there was an activation of a cortical network similar to that found in adults, including the premotor cortex, the posterior part of inferior frontal gyrus and posterior parietal lobe. However, differently from our study, Biagi and coworkers showed a more bilateral activation. Indeed, the activated network was more lateralized to the left hemisphere in adults and more bilateral in children, with a linear increase of lateralization index as a function of age. The discrepancy with the present findings could be explained by the type of stimuli used for the fMRI tasks, which consisted of video-clips of hand actions performed with the right or left hand in the study by Biagi, and grasp-to-place actions performed only with the right hand in the our study.

Modulation of the MNS in UCP children: the role of motor experience

Few studies have explored the neural basis of action observation in CP children using neuroimaging techniques (Dinomais et al. 2013a; Dinomais et al. 2013b). However, these studies were not carried out on homogeneous groups and did not take into account motor impairment level, lesion side, or specific hand kinematics. A study carried out by Dinomais and coworkers (2013a) showed a preserved motor cortical activation during observation of simple opening-closing hand movements, regardless of degree of motor impairment. Differently from our study, they did not use, as video stimuli, goal-directed hand actions. In the present fMRI study, focused on observation of reaching-grasping actions in a specific cohort of UCP children, the data showed, both during observation of paretic and healthy hand, a bilateral pattern of activation of the MNS, although this activation was stronger in the right, contralesional hemisphere. The most important finding was the greater activation found in UCP group, during observation of a grasping action performed by a paretic hand with respect to observation of the same action performed by a healthy one, both in PPC and PMC at a individual and group level, based on ROI analysis.

These findings could, in principle, be attributed to basic features of employed stimuli or to unspecific factors. One of these features could be the effective duration of the observed hand movement, which was dissimilar between the two conditions. However, the absence of a differential activation in TD children indicates that this explanation can be excluded. Attentional load could, in principle, constitute another distinction between the two groups, however results of behavioral analysis did not reveal any statistical difference in accuracy during catch trials between TD and UCP children. Another possible confounding factor could be the type of grip used by the paretic and healthy hand model. However, in TD participants the activations in the two conditions were very similar, thus it is unlikely that this factor could influence the main result. Finally, the present findings cannot be explained by visual familiarity with the observed model. In fact, the paretic model is unfamiliar to TD children, but activation within ROIs was not different between the two conditions, while children of UCP group have visual familiarity with both models, but brain activation was higher for the paretic hand condition.

The modulation of brain activity found in UCP children in the two action observation conditions appears to be better explained by the property of the MNS to reflect the individual’s own motor repertoire, confirming the main hypothesis underlying this investigation. This explanation is in line with several neuroimaging studies on healthy participants (Buccino et al. 2004a; Calvo-Merino et al. 2005, 2006; Haslinger et al. 2005; Cross et al. 2006) indicating a strong role of MNS in representing previously acquired motor skills. For example, some fMRI studies have shown that in participants with specific motor expertise (dancing, playing basketball, playing piano etc.), the observation of actions belonging to their own motor repertoire (Calvo-Merino et al. 2005, 2006; Haslinger et al. 2005; Abreu et al. 2012) determine a stronger activation of the parieto-frontal MNS when compared with activation elicited in non-expert controls. This leads one to suggest that, in UCP children, cortical motor representation of the affected hand better matches the visual description of the paretic hand model. One could argue that, in daily life, they also have the experience of their unaffected hand. However, the hand presented in the action observation conditions (either paretic or healthy) is always the right one in a first-person perspective, thus anatomically corresponding to the affected hand of the enrolled UCP children.

In TD children, contrary to UCP ones, BOLD response in PPC and PMC regions of the left hemisphere during observation of paretic versus healthy hand was very similar. This could depend on the fact that for TD participants the pathological model is a kind of simplification of their hand motor representation. Thus, when they observed a pathological model, their motor system matched the goal of the action more than the way in which it was executed. This interpretation is in line with studies showing that observation of actions performed by artificial (e.g. robot) or by biological agents elicited similar MNS activation (Tai et al. 2004; Gazzola et al. 2007). Interestingly enough, after the experiment, some TD participants reported no evident differences between the two observed models.

Representation of action goals and movement kinematics in UCP children

In the present study, observation of grasp-to-place actions enhanced activation of posterior parietal and premotor areas in UCP children, consistent with previous studies on action observation (Buccino et al. 2001; Gazzola et al. 2007; Biagi et al. 2010; Caspers et al. 2010; Molenberghs et al. 2012; Gatti et al. 2017). However, in the majority of studies on action observation, it is difficult to disentangle the processing of action goal from action kinematic components. For example, Gazzola and coworkers (2007), in an fMRI study, asked normal adult participants to observe videos displaying a human or robotic arm/hand grasping objects, showing that, despite shape and kinematic differences, the parieto-premotor MNS became equally active in both conditions. As a matter of fact, few studies have directly compared activations produced by different action goals or different kinematic features. Among these latter studies, those of Hamilton and Grafton (2006, 2008) used fMRI repetition-suppression technique to identify possible areas involved in the processing of different features of simple hand actions (e.g. push-pull or grasp). The results suggest that action goal is encoded in the posterior parietal and premotor areas belonging to the MNS, while basic kinematic features of an action (e.g. type of grip, trajectory) are processed by occipital, superior precentral and middle frontal areas. On the other hand, other studies indirectly showed that areas belonging to the parieto-frontal MNS process not only the goal of the observed action, but also the way to achieve it. These studies have revealed stronger responses to human than animal actions (Buccino et al. 2004a), to actions within the motor repertoire than those outside it (Calvo-Merino et al. 2005, 2006), to human agents than robotic agents (Tai et al. 2004; Shimada 2010) or to other non-biological effectors (Costantini et al. 2005). Thus, the stronger activation can be explained in terms of motor repertoire closer to that of the observer, but this is not conclusive with regard to a possible different role of the action kinematic components.

In an action observation-based rehabilitation therapy both action goal and kinematic features must be considered. Clinical reports during AOT application (Sgandurra et al. 2013) indicate that observation of healthy models elicits, in UCP children, a marked propensity, during movement reproduction, to preserve task proficiency, by selecting those movements which guarantee final action outcome. For example, in order to grasp an object, UCP children do not try to imitate the typical kinematics of a healthy hand, but rather adopt motor strategies most suitable in achieving their goal. This choice is probably induced by the notable differences between UCP movement kinematics and observed healthy ones. On the contrary, it is plausible that when observed actions are performed by a hand kinematically similar and anatomically corresponding to their affected hand, UCP children tend to show a stronger activation of the MNS, which in turn could induce a motor reproduction more similar to the observed model. Thus, at different processing levels, both the action goal and plausibility of the means used for its execution can be important for these children. This interpretation is corroborated by previous studies on healthy individuals. For example, in a study in which repetitive Transcranial Magnetic Stimulation (rTMS) was applied to the ventral premotor cortex during observation of biologically possible and impossible motor acts, it has been shown that the premotor sector is crucial for discriminating these two different categories of acts (Candidi et al. 2008). Moreover, in an fMRI study, Casile and coworkers (2010) demonstrate that regions in the left dorsofrontal and dorsal premotor cortex are differentially activated by observed human movements dependent on their compliance with the kinematic invariants of human movements (two-thirds power law). Hence, in their study, motor resonance with observed movements seems to be critically modulated not only by the appearance of the agent and by the action goal, but also by the consistency with the human kinematic laws of motion. This supports the notion that the MNS is important for analyzing the goal of the observed action on the basis of its biologically possible kinematics. Thus, it is plausible that, also in UCP children, the premotor and parietal cortex are sensitive to observed action kinematics. In situations in which the observed action is not within the personal motor repertoire (e.g. healthy hand for UCP children), deviation in kinematics could be an important element for explaining the lower motor resonance with the observed model.

Correlation between type of lesions and functional reorganization of the MNS

In humans, as in other mammals, corticospinal projections, early in development, produce transient ipsilateral projections, most of which are eliminated when maturity is reached (Eyre et al. 2001, Eyre 2007). In neonates, for example, focal TMS of the motor cortex evokes ipsilateral responses with similar threshold and amplitude as obtained for contralateral responses; this indicates a bilateral innervation of the spinal cord from motor areas of both hemispheres (Eyre

2007). Longitudinal studies on healthy infants showed a consistent withdrawal of ipsilateral corticospinal projections, while contralateral projections are enhanced (Eyre et al. 2001). Functional and anatomical evidence in animal models and in humans have demonstrated that such process depends also on environmental and experience factors (Martin et al. 2004; Martin 2005; Friel and Martin 2007).

In congenital brain damage leading to CP, different types of brain reorganization can be observed. The main mechanism for reconnecting the brain to spinal cord is a reorganization that occurs within the ipsilesional cortex, in regions inside the primary motor cortex (M1) or non-primary motor areas (Boyeson et al. 1994; Donoghue et al. 1996; Hallet et al. 2001). In other cases, when the lesion occurs at an earlier stage of life, either during intrauterine life or soon after birth, a different mechanism can also be observed (Carr et al. 1993; Staudt et al. 2002, 2004; Guzzetta et al. 2007), in which a significant number of monosynaptic fast conducting ipsilateral projections from the undamaged cortex persists (contralesional reorganization). This mechanism allows the undamaged cortex to directly control both upper limbs (Staudt et al. 2004; Guzzetta et al. 2007). Staudt and coworkers (2002, 2007) detected a strikingly clear relationship between the extent of the periventricular lesion and type of motor reorganization: patients with small lesions showed preserved ipsilesional (contralateral) projections to the paretic hand, whereas a majority of patients with large lesions have fast conducting ipsilateral pathways originating in the contralesional hemisphere. This could suggest that both lesion extent and different mechanisms of cortical reorganization associated with upper limb impairment may be correlated to the activation of the MNS during action observation or other tasks, such as motor imagery for grasping actions. Moreover, this allows us to make hypotheses on the possible impact of AOT for upper limb rehabilitation in children showing different reorganization mechanisms.

The UCP patients enrolled in this study presented lesions involving the periventricular area, with the greatest lesion overlap in subcortical white matter of the left hemisphere. Conversely, cortical involvement of regions outside the periventricular zone, i.e. inferior frontal, dorsolateral frontal, inferior and superior parietal regions, was much less frequently found (N = 3 subjects). The use of the MNS Localizer allowed us to observe an activation of PPC and PMC also in the damaged hemisphere, similarly to the findings of the action observation task, in five UCP children (subjects #2, #3, #4, #9, #10). Contrarily, in the remaining UCP patients (subjects #1, #5, #6, #7, #8), activation clusters were found mainly in the right contralesional hemisphere. Thus, although the case group was relatively homogeneous in terms of degree of upper limb impairment and lesion side, patients showed different motor system reorganization patterns, with respect to hemispheric lateralization of activation. In the first group, the lesioned hemisphere could still have some control over the affected hand, while in the second one this control was probably shifted to the contralesional, undamaged hemisphere. Although we did not directly test activation during movement execution with affected hand, the MNS Localizer task, consisting of motor imagery of affected hand, gave results consistent with this prediction. Of course, this finding is not conclusive, but it seems to corroborate the evidence provided by current literature on brain reorganization in UCP children (Staudt 2007; Reid et al. 2015) and may allow to create an anatomo-functional link between cortical representation of actions during action observation and motor reorganization.

Further experiments combining neuroimaging and electrophysiological techniques (e.g. TMS) could enable to directly correlate brain activation during observation with cortical pathways used for motor control.

A different model of AOT as a tool for motor rehabilitation

Recent studies support the hypothesis that action observation drives reorganization of motor representations to form new motor memories of observed actions (Classen et al. 1998; Stefan et al. 2005, 2008). On this basis, AOT has been used in the rehabilitation of neurologic patients, both adults and children (Sale and Franceschini 2012; Buccino 2014; Sarasso et al. 2015). However, a better understanding of the underlying mechanisms could lead to further improvements of the effects of observation-based therapies. The present study suggests that observation followed by imitation of a model whose motor skills are more closely matched to those of the observer could lead to a greater motor improvement. In fact, if motor recovery in UCP patients were possible on the basis of imitation of a healthy model, they could simply copy their unaffected hand. In addition, the use of different AOT models, based on the degree of motor impairment, could allow this therapy to be adapted to the upper limb clinical characteristics of each individual patient. To better define which patients would benefit most from observation therapy based on a pathological model, future studies should be designed as randomized controlled trials (RCT) in order to evaluate, under homogeneous clinical conditions, efficacy of AOT based on a pathological model to improve upper limb activity in UCP children.

General Discussion

The mirror mechanism and the cognitive functions attributed to the cortical motor system open new scenarios in the clinical domain offering a novel basis for developing new strategies in the rehabilitation field. The AOT is an example of rehabilitation approach who has possibly as a main target the MNS. This especially because the mirror mechanism may account for our capacity to resonate with observed actions and to learn through imitation. Using fMRI, the main aim of the present thesis was to understand how the motor expertise modulates the MNS activation, in order to adapt action observation to the patient's upper-limb clinical characteristics and improve motor recovery. In the presented studies, we investigated the functional properties of MNS areas: 1) in healthy humans observing hand actions performed by other individuals with different levels of motor expertise; 2) in children with UCP observing reaching-grasping actions performed by another hemiplegic child or by a healthy child.

The experimental evidence presented above corroborates the concept that the role of the motor system is not limited to mere executive functions. The motor system, in fact, is provided with the ability to resonate when actions, that are already or could become an integral part of our motor repertoire, are observed. This resonance mechanism allows one to understand actions made by other people and to capture the intentions of the agent. In the first study, it has been demonstrated that healthy naïve individuals without particular motor skills tend to resonate stronger during the observation of hand manipulative actions performed by a novice model, compared to actions performed by an expert or an intermediate model. This suggest that if the observed model executes actions already embodied in the personal vocabulary of the observer, it will be able to produce a stronger activation of the MNS compared to the observation of the expert model. Another important finding was that, even if our group of healthy participants was homogeneous in terms of motor skill, it was different in terms of small deviations in participant's hand motor dexterity. Indeed, motor ability is not an all-or-nothing concept but identifies different levels of expertise that could potentially influence action perception, as during observation. This is a new concept in neuroimaging studies on expertise. In fact, previous evidences reported that experts show an increase in activation within the MNS during observation of actions similar to their expertise (Calvo-Merino et al. 2005, 2006; Halsinger et al. 2005; Cross et al. 2006) compared to novices, in which no expertise-related effect was found. In our study there was a modulation in brain activation, in particular within the dorsal fronto-parietal MNS/AON, considered involved in the processing of kinematic aspects of the observed actions. Moreover, an interesting result was that dorsal areas, namely the SPL and PMd cortex, are not only anatomically interconnected, but also functionally coupled during the observation of actions executed with a level of expertise similar to that of the observer.

One possible issue is whether our findings result from either visual familiarity or motor familiarity with stimuli. In our opinion, the increased responses of the dorsal MNS/AON in healthy individuals observing a naïve model compared to the expert one may reflect motor resonance. We suggest, first of all, that participants did not have visual familiarity with the manipulative actions selected for the action observation task, but they had motor familiarity with the novice model; second, we observed expertise effects in the SPL and the PMd cortex, which are considered as sensorimotor areas; third, previous studies demonstrated that dancers who trained regularly showed stronger activation in the MNS when they watched movements performed by dancers of their own gender, whereas there was no increase in activation when they watched movements performed by dancers of the opposite gender, which they frequently saw but did not perform (Calvo-Merino et al. 2006).

Our results could be very relevant for the improvement of motor learning in healthy individuals and patients with motor impairments using action observation and imitation. Ten years ago, it was proposed that the careful observation of actions, followed by imitation (AOT), would become a valid approach in motor rehabilitation (Ertelt et al. 2007), since even action observation has proven to be effective in recruiting motor representations of observed actions (Fadiga et al. 1995; Stefan et al. 2005, 2008). Typically, in AOT, patients who had a reduction of motor skills are required to carefully and systematically observe, during a rehabilitation therapy that lasts 3 to 4 weeks, a series of movies that display everyday actions (drink coffee, read the newspaper, clean the table, inserting a card into a slot etc.).

Here, in the second study described in this thesis, we deeply explored the mechanisms underlying action observation and motor resonance in UCP children, in order to achieve a better knowledge about the effects of observation-based therapies. Our results suggest that observation followed by imitation of a model whose motor skills are more closely matched to those of the observer could lead to a greater motor improvement. This also suggests the possibility to adapt the AOT to the individual upper limb clinical characteristic in order to improve motor function in hemiplegic children as well as in other neurological patients. Based on the individual motor repertoire it could be possible to enhance the activation of the MNS using a model kinematically similar to that of the observer. Furthermore, the main clinical characteristic of hemiplegia certainly is the reduction of the motor repertoire of the affected hemi-side in module acquisition (meant as the elementary components of movement the child is provided with), combinations (possibility to subdivide the individual modules into different patterns according to space relations), and sequences (ability to assemble the individual modules according to different time relations). On this assumption, it is possible to hypothesize that hemiplegic children, with activation of the MNS despite the presence of extended lesions, could resonate strongly during the observation of another hemiplegic child. Here we confirm this hypothesis, and the present findings are in line with previous studies, that have reported an experience-dependent modulation of the motor system for actions belonging to the own motor repertoire (Calvo Merino et al. 2005, 2006; Haslinger et al. 2005; van Elk et al. 2008). We described also a possible neurophysiological mechanism underlying this motor resonance possibly based on the activation of the dorsal MNS/AON encoding the detailed representation of the actions, differently from the ventral one, processing the general goal of the action.

The adaptation of the AOT to the patient’s individual motor repertoire is an approach that finds its neurophysiological bases in the property of MNS of mirroring the personal motor experience and finds also a correspondence in the educational science field. Several years ago, Lev Semenovich Vygotsky (1896-1934) proposed the concept of zone of proximal development (ZPD) defined as “the distance between the actual development level and the level of potential development as determined through problem solving under adult guidance or in collaboration with more capable peer” (Vygotsky 1978). That is, the ZPD was described by Vygotsky as actual level of development of the learner and the next level attainable through the use of mediating environmental tools and capable adult or peer facilitation. The idea is that individuals learn best when working together with others during joint collaboration, and it is through the imitation of more skilled individuals with similar ability. This suggests that UCP children with a poor motor repertoire could benefit from rehabilitation strategy based on action observation and imitation of another child performing actions that are not part of his motor vocabulary. However, the model is required to be similar to the learner. In fact, if motor recovery in UCP patients were possible on the basis of imitation of a healthy normal model, they could simply copy their unaffected hand. On the contrary, the use of different AOT models, based on the degree of motor impairment, could allow this therapy to be adapted to the upper limb clinical characteristics of each individual patient. Finally, new studies should be designed as RCT in order to define which patients could improve their motor skills through action observation and imitation and to better define the characteristics of the best model for AOT.

Supplementary Material

Supplementary Table 1: Mean scores and Equivalent scores calculated for each test used to assess hand motor ability in the three actors performing the manipulative actions. M = Mean; Z = standardized Z Score; MFT = Maximum Finger Tapping Frequency.

	Pardue Pegboard Test		MFT frquency		Minnesota Dexterity Test	
	M	*Z*	*M*	*Z*	*M*	*Z*
Novice	14	-0.10	170	0.50	192 s	1.82
Intermediate	19	2.11	182	1.10	178 s	2.54
Expert	23	4.13	211	2.56	154 s	3.32

Supplementary Table 2: Standardized Z scores obtained by participants assessed with the PPT.

N	*Right Hand (Z)*	*Right Hand (Z)*	*Bimanual (Z)*
# 1	-0.80	-0.81	0.48
# 2	-0.03	-0.89	0.48
# 3	-0.61	-0.21	2.51
# 4	0.03	0.41	1.33
# 5	0.55	1.33	1.60
# 6	-0.15	0.19	1.50
# 7	0.67	0.43	1.65
# 8	1.26	-2.24	0.21
# 9	-0.61	-0.71	1.24
# 10	-0.18	0.08	0.55
# 11	-0.16	0.21	1.13
# 12	-1.02	-1.45	-1.78
# 13	-1.67	-2.29	0.48
# 14	-0.37	-0.57	-0.87
# 15	-0.58	0.21	0.81
# 16	0.39	-0.04	-0.05
# 17	1.15	1.12	1.14
# 18	1.24	1.11	1.13

Supplementary Table 3: GLM group analysis results during (**A)** *AO Novice* vs. *AO Ctrl;* (**B**) *AO Intermediate* vs. *AO Ctrl;* **(C)** *AO Expert* vs. *AO Ctrl*. Local maxima, as shown in Figure 1, are given in MNI standard brain coordinates, significant threshold set at P_{FWE} <0.001 (cluster-level).

Anatomical region	Left Hemisphere				Right Hemisphere			
	x	y	z	Z-score	x	y	z	Z-score
(A) AO Novice vs. AO Ctrl								
Inferior Parietal Lobule (PFt)	-50	-22	32	7.10	62	-22	30	6.12
Cerebellum (LobuleVI)	-28	-54	-18	5.96	28	-54	-20	6.73
Precentral gyrus	-30	-12	64	6.51	28	-10	58	6.14
Insula	-38	-6	14		38	0	11	6.33
Cingulate Cortex	-6	-30	24	5.15	6	-22	26	6.06
Intraparietal Sulcus	-34	-54	56	3.70	40	-44	50	4.38
Thalamus					26	-26	0	4.88
IFG (pars Triangularis)					40	32	16	4.63
IFG (pars Opercularis)					58	14	32	4.51
Fusiform gyrus	-30	-52	-14	6.25	34	-48	-14	6.47
Superior Parietal Lobule	-32	-46	64	6.16	34	-52	60	5.51
Postcentral Gyrus	-38	-38	56	6.07	32	-36	52	6.14
(A) AO Intermediate vs. AO Ctrl								
Inferior Parietal Lobule (PFt)	-50	-22	32	6.53	53	-20	30	5.32
Cerebellum (LobuleVI)	-28	-50	25	6.51				
Precentral gyrus	-30	-14	62	6.09	30	-10	58	5.10
Cingulate Cortex	4	-28	30	6.17				
Middle Frontal Gyrus					48	40	22	3.82
IFG (pars Triangularis)					48	22	24	3.40
Thalamus					12	-22	-8	3.42
Fusiform gyrus	-30	-50	-14	6.46	30	-48	-14	7.08
Superior Parietal Lobule					34	-52	60	4.44
Postcentral Gyrus	-38	-38	58	5.20	32	-36	52	5.37
Precuneus	-14	-64	34	5.08	14	-70	40	5.88
(A) AO Expert vs. AO Ctrl								
Inferior Parietal Lobule (PFt)	-64	-26	34	4.67	54	-22	30	4.56
Cerebellum (LobuleVI)	-28	-50	-24	4.81	26	-54	-20	4.96
Precentral gyrus	-30	-12	64	4.40				
Insula					38	0	12	3.96
Cingulate Cortex	-4	-20	30	4.64	6	-14	30	4.76
IFG (pars Triangularis)					40	34	12	4.43
Fusiform gyrus	-30	-52	-14	4.62	26	-62	-10	5.33
Superior Parietal Lobule					34	-52	60	3.57
Postcentral Gyrus	-42	-30	42	4.21	50	-24	46	3.55
Precuneus	-14	-65	34	3.54	12	-66	36	4.65

Supplementary Table 4. Brain areas showing differential activation for the main effect of type of observed model (AO Novice vs. AO Expert). Only regions surviving a cluster-level threshold of P < 0.001 are reported. Coordinates correspond to local maxima of the respective clusters, and are defined in Montreal Neurologic Institute stereotactic space.

Anatomical region	Left Hemisphere				Right Hemisphere			
	x	y	z	Z-score	x	y	z	Z-score
AO Novice vs. AO Expert								
Superior Parietal Lobule	-14	-56	66	4.81	16	-54	64	5.28
Precentral Gyrus	-32	-8	60	3.98				
Inferior Parietal Lobule	-50	-20	32	4.34				
Intraparietal Sulcus					34	-40	44	3.96
Superior Frontal Gyrus	-22	-10	52	3.93				
Inferior Occipital Gyrus	-22	-86	-4	4.37	30	-85	-2	4.66
Thalamus					26	-26	-2	4.57
Fusiform Gyrus	-37	-70	-14	4.38				
Middle Temporal Gyrus	-50	-66	-2	4.23				

Supplementary Table 5. PPI activations. Brain areas involved in a psycho-physiological interaction with SPL seed region during the observation condition *AO Novice* vs. *AO Expert,* thresholded at $P_{FWE} < 0.005$, cluster level corrected. Local maxima, as shown in Figure 7, are given in MNI standard brain coordinates.

Anatomical region	Left Hemisphere				Right Hemisphere			
	x	y	z	Z-score	x	y	z	Z-score
(A) AO Novice vs. AO Ctrl								
Superior Parietal Lobule	-	-	-	-	38	-52	58	3.99
Intraparietal Sulcus	-36	-48	48	4.20	32	-46	48	4.34
Precentral Gyrus	-30	-10	58	4.11				
Postcentral Gyrus	-34	-34	42	3.72				
Inferior Parietal Lobule (PFt)								
Cerebellum (Crus 1)	-40	-60	-26	4.18	34	-82	-20	4.57
Middle Temporal Gyrus					52	-58	-2	4.21

Supplementary Table 6: GLM group analysis results for UCP children during (**A)** *AO Paretic Hand vs. rest;* (**B**) *AO Healthy Hand vs. Rest*. Local maxima, as shown in Supplementary Figure 1, are given in MNI standard brain coordinates, significant threshold set at P_{FWE} < 0.001 (cluster-level). *Lesioned Hemisphere

Anatomical region	Left Hemisphere*				Right Hemisphere			
	x	y	z	Z-score	x	y	z	Z-score
UCP CHILDREN								
(A) AO Paretic Hand vs. Rest								
Middle Occipital Gyrus	-22	-94	6	5.71	28	-84	8	6.46
Superior Occipital Gyrus	-16	-86	14	5.74	24	-92	14	5.92
Middle Temporal Gyrus	-44	-62	6	5.98	42	-70	0	5.37
Superior Parietal Lobule	-24	-54	56	3.68	28	-62	60	5.28
Superior Frontal gyrus					28	-14	60	4.51
Postcentral gyrus	-40	-36	52	3.74				
Precentral gyrus	-50	-2	34	3.84	58	2	46	4.65
Inferior Parietal Lobule	-54	-30	40	3.35	30	-52	50	5.03
Inferior frontal gyrus					50	26	22	3.95
Thalamus					22	-26	-8	4.95
(B) AO Healthy Hand vs. Rest								
Middle Occipital Gyrus	-20	-94	6	6.05	28	-84	8	6.51
Superior Occipital Gyrus					24	-92	14	6.24
Middle Temporal Gyrus	-42	-62	4	5.64	56	-50	8	4.49
Superior Frontal gyrus					50	36	30	3.90
Superior Parietal Lobule	-24	-56	58	3.48				
Intaparietal sulcus					-34	-44	44	4.93
Precentral gyrus	42	6	42	3.67	58	2	46	4.31
Thalamus	-24	-24	12	4.96	22	-26	-8	5.10

Supplementary Table 7: GLM group analysis results for TD children during (**A)** *AO Paretic Hand vs. Rest;* (**B**) *AO Healthy Hand vs. Rest*. Local maxima, as shown in Supplementary Figure 1, are given in MNI standard brain coordinates, significant threshold set at $P_{FWE} < 0.001$ (cluster-level).

Anatomical region	Left Hemisphere				Right Hemisphere			
	x	y	z	Z-score	x	y	z	Z-score
TD CHILDREN								
(A) AO Paretic Hand vs. Rest								
Middle Occipital Gyrus	-22	-92	13	7.60	30	-92	16	5.97
Middle Temporal Gyrus	-48	-60	6	5.03	48	-44	10	3.86
Inferior Temporal Gyrus	-48	-74	-2	5.99	52	-66	-4	6.68
Superior Parietal Lobule	-20	-60	66	6.18	24	-60	58	5.37
Postcentral gyrus	-64	-6	24	4.58				
Inferior Parietal Lobule	-40	-38	44	4.52				
Intraparietal sulcus	-22	-56	52	5.58	30	-42	42	4.57
Precentral gyrus	-48	-6	56	5.31	42	-2	50	4.44
Thalamus					18	-30	-2	4.58
Hippocampus					24	-26	-10	4.25
(B) AO Healthy Hand vs. Rest								
Middle Occipital Gyrus	-22	-92	16	7.37	30	-92	18	5.29
Middle Temporal Gyrus	-48	-60	6	4.27	48	-44	10	3.76
Inferior Temporal Gyrus	-48	-74	-2	5.11	52	-66	-4	5.31
Superior Parietal Lobule	-22	-56	50	5.20	24	-60	58	4.66
Inferior Parietal Lobule	-40	-40	42	4.42				
Intraparietal sulcus	-22	-56	50	5.20	32	-40	42	3.95
Precentral gyrus	-48	-6	56	5.17	42	-2	50	3.69
Caudate Nucleus	-14	26	0	4.01	16	22	0	4.69

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